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Editor

HAMILTON B. G. ROBINSON

DECEMBER, 1952

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Myers, Hamilton, and Becks (p. 743) resorted to the use of radioactive fluorine in an effort to explain the mode of action of topically applied fluorides. This interesting study, using F^{18} which has a short half life, demonstrated that only 2 per cent of the fluoride ions on a cotton pledget are transferred to the tooth during topical application, and that the major portion of the fluorides which are transferred to the teeth are removed by rinsing. The fluorides were concentrated in surface defects and discolored enamel and usually the areas of fluoride concentration could be demonstrated either by roentgen or Grenz rays or by infrared or ultraviolet photography. The results of this study suggest that the protective action of topical fluorides are related to their uptake in imperfections rather than to general surface adsorption.

Fischer and Muhler (p. 751) studied the effect of sodium fluoride on the surface structure of powdered dental enamel. They demonstrated that sodium fluoride immersion results in a direct transformation from an apatite to a calcium fluoride on the surface of powdered enamel. The pH of the solution had a marked effect on the change to calcium fluoride, as also did the concentrations of the fluoride solution and the time of treatment. These effects on powdered enamel surfaces might explain the manner in which fluorides penetrate the defects in the enamel suggested in studies using radioactive fluorides but the intact enamel surface may not react in the same way as powdered enamel.

Muhler, Day, and Nebergall (p. 756) used freshly prepared stannous fluoride solutions and aged stannous fluoride solutions in comparison with fresh and aged sodium fluoride solutions. It was found that enamel picks up larger amounts of fluorine from stannous fluoride regardless of age of the solution, but that the effectiveness of stannous fluoride in reducing the solubility of powdered dental enamel decreases with age of the solution. These in vitro findings indicate the necessity for clinical evaluation of stannous fluoride solutions against sodium fluoride solutions for topical application in control of dental caries. The final test is whether the stannous fluoride solutions, whether fresh or aged, are more effective in reducing dental caries than sodium fluoride solution, and until they have been evaluated, clinically, their general use should not be encouraged.

The problem of the modus operandi of fluorine in reducing dental caries activity has stimulated much speculation and some worth-while research. Rathje (p. 761) reviews some of the literature on the action of fluorine in caries control and reports on the reduction of hyproxyapatite and fluor-apatite experimentally. Using hydroxy-apatite and fluor-apatite mixtures in different mouths, it was demonstrated that the solubility of hydroxyfluor-apatite is considerably influenced by very small changes in the fluorine content. Rathje discusses the probability that hydroxyfluor-apatite may originate in the enamel by the influence of drinking water containing fluorides. If hydroxyfluor-apatite does occur the enamel should become less soluble and, therefore, be more resistant to dental caries. One suggestion of Rathje's is that further studies of fluorine enamel relationship be made by use of radioactive fluorides. Another paper in this issue (p. 743) discusses this subject.

It is important that the method of action of caries-inhibiting agents be understood in order that any useful properties might be augmented by substituting more effective agents to perform the given functions. Schneyer's study (p. 767) makes it appear unlikely that high ammonia-urea dentifrices act by denaturing amylase. When one type of therapeutic dentifrice appears more effective than a similarly constituted dentifrice, it is essential to consider the possible activity of all agents in the dentifrices.

Many varying results have been reported with the use of ammonium ion tooth powders in the control of dental caries. In a study by Davies and King (previously reported in the JOURNAL OF DENTAL RESEARCH) comparisons were made with a dentifrice which was assumed to be the same as that used in the experimental study of Kerr and Kesel (*J. A. D. A.* 42: 180, 1951). However, Davies and King's attention has been called to the fact that the dentifrices which they used were not identical with those which were used by Kerr and Kesel and they report these differences in the present issue of the *Journal* (p. 775). If small differences in the dentifrices and the composition of ammonium ion in dentifrices lead to a large difference in experimental results, this particu-

lar factor should be investigated thoroughly.

Diazotization was used by Burnett and Scherp (p. 776) as an indicator of accessibility of dentin for destruction by proteolytic enzymes. This study was undertaken because, although it has been claimed by some investigators that caries is primarily enamel matrix and dentin, these organic structures do not appear readily accessible for bacterial destruction. In preparing the dentin it was found that at the same pH different acids could decalcify dentin at different rates. Dentin decalcified in acids and naturally carious dentin reacted in varying degrees to diazohydroxide. Noncarious dentin does not react readily to diazohydroxide nor is it readily available for lysis by proteolytic enzymes. The results do not appear to lend support to the theory that caries is initiated by proteolytic breakdown of organic material.

The amino acid content of dentinal protein has been investigated by Hess, Lee, and Neidig (p. 791). They determined the presence of 20 different amino acids which accounted for 8 per cent of the total nitrogen of the dentin. In the concentrations found, they concluded that dentinal protein is a collagen. At the same time, Scott (*J. D. Res.* **31**: 484, 1952) reported findings under the electron microscope which also appear to show that dentinal protein is a collagen. It is interesting to review the methods used by the biochemical investigators and the microscopic investigators. The methods of chemical analysis included the use of bacterial forms, as well as chemical agents. Likewise, the microscopic investigator is no longer limited by the conventional microscope, but may venture beyond the limits of the optical system, using electron microscopes, polarized light, and x-ray diffraction.

Pincus had reported extremely high yield from dentin or chondroitin sulfuric acid. Hess and Lee (p. 793) repeated the analysis with his, and other techniques, obtaining much lower values within the range of theoretical analysis. They have shown that the chondroitin sulfuric acid from dentin resembles that from cartilage (bovine tracheal). There is often a willingness to accept reported data, without confirmation, in the desire to advance knowledge as rapidly as possible. While this enthusiasm is commendable, the older scientific approach of rechecking and repeating has great merit. This paper emphasizes the need for such confirmatory studies.

A strain of rats in which the females have less decay "biologically" than the males was used by Muhler and Shafer (p. 798) in caries-sex hormone studies. Orchiectomy reduced caries in the males and testosterone appeared to lower caries activity still more in these animals, although the hormone increased decay in the control males. On the other hand, ovariectomized rats had a statistically insignificant reduction in caries activity and administration of diethylstilbestrol significantly increased decay in the females, while it reduced caries in control females. This confusing picture does not permit definite conclusions but suggests interrelationship of hormones that may influence caries in the rat. Whether their effect is direct or indirect is not evident but indirect effects of testosterone and diethylstilbestrol is suggested by the results.

Pigmentation failure of the incisors of rats on vitamin E deficient diets has been reported many times. Pindborg (p. 805) studied the histologic changes (cyst formation) in the enamel organs of vitamin E deficient rats and traced the cause to edema of the papillary layer. The edema probably is a result of capillary damage. Vitamin E also seems to effect capillary permeability in the rat.

Pindborg (p. 812) has confirmed the observation of Aaes-Jorgensen, Dam, and Granados that Antabuse (tetraethylthiuram disulfide) can substitute for vitamin C in preventing loss of pigmentation and histologic changes in the teeth of rats. The mechanism is not explained.

Irving, Pindborg, Fitzhugh, Weinmann, and Schour (p. 815) studied the effects of sodium sulfite on the incisor of the rat. Sodium sulfite is used as a preservative in foods, particularly in dried fruits fed to troops in warm climates. The studies of the rat incisor showed that sodium sulfite resulted in deficiencies in vitamin E and vitamin A. The rat incisor is highly sensitive to subclinical vitamin deficiencies and is extremely useful in assaying vitamin deficiencies. As pointed out by the authors, there are certain changes in and around the rat incisor which are typical for deficiencies for some of the vitamins.

Being in a continuously erupting tooth, the changes observed are those of rapid growing embryological type tissues which have a much greater sensitivity to injury.

If salivary bacterial indices are to be used for estimation for caries activity, they should be re-evaluated and improved from time to time. Rizzo and Tilden (p. 825) report a comparative study of the tomato agar count, the Snyder test, and the acidogenic count (Davis, Schlack, Tilden), with the clinical progress of caries. They confirmed the association of caries activity with the presence of a large number of salivary lactobacilli, but found that streptococcus counts were uniformly high in children irrespective of clinical conditions. In a few cases, they found caries developing in the apparent absence of lactobacilli. They suggest that counts of lactobacilli from bacterial plaques should be made in such cases. In these cases, it is also possible that there are highly acidogenic lactobacilli which are present in the saliva and which would be found in plating of nondiluted saliva, or that the lactobacilli which are present are not able to adapt themselves to the media employed.

Tilden and Svec (p. 831) found that the predominant species of lactobacilli in the saliva of children was *L. casei* which is strongly acidogenic. *Lactobacillus acidophilus* was isolated from only a small percentage of children and is not particularly acidogenic. Other lactobacilli isolated included *L. fermenti* and lactobacilli which resembled *L. casei* but which are unable to ferment lactose. The species of lactobacilli which are present in saliva have not received the attention that one would expect and further differentiation of the lactobacilli from susceptible and immune individuals should give useful information to those who are interested in dental caries control.

Fulton (p. 839) used data from health examinations in the state of Illinois and from the Cleveland Board of Education to determine dental caries experience rates in primary teeth. One of the interesting findings is the very small number of deciduous teeth which are filled in comparison with the number decayed, or missing. The Illinois preschool children have a much higher rate of fillings and an appreciably lower *def* rate than do the Cleveland children in this particular study. The possible significance of this is discussed.

Examination of the mouths of 1,954 students in Utah by Wilcox, Greenwood, and Galloway (p. 844) showed significant differences betwen the students from Utah and those from out of state. It was impossible to correlate the variations in caries experience with reported dietary habits. The Utah students had a higher DMF rate than students who had studied in Oregon, and children in Hagerstown, Md. However, it should be pointed out that it is extremely difficult to compare studies made in different parts of the country under different conditions. The method of the dental examination may result in differences in caries experience records in two different areas of the country. The evaluation of students from Utah and those from out of the state examined by the same examiners are much more significant than comparisons of the studies by different examiners.

Wilcox, Greenwood, and Galloway (p. 849) report the results of examination of a group of 264 children with rheumatic fever and compare the DMF rates of these children with those of children without rheumatic fever. Comparison of the dental caries experience of the normal and rheumatic fever children shows no significant differences. The authors point out that the caries experience of all of the children examined was relatively high when compared with children in other studies. It is important that the children used in the present study were given dental examinations, including posterior bitewing radiographs, in dental offices. Here, the facilities are at the optimum for identification of carious lesions and it would be expected that the rates would be higher than among those who are examined in large groups with limited facilities.

Loewenstein and Faivovich (p. 854) have approached the problem of the diagnosis of pulpitis with electrical current in a different manner than that which is commonly used. They have studied the excitability of dental nerve tissue in relation to time. Their studies show some interesting variations from tooth to tooth in different mouths and also on the same type of tooth in different mouths. It is interesting that the excitability of normal teeth and of those with pulpitis was not significantly different. This is a highly important problem in relationship to clinical diagnosis. The authors point out that pulpitis is a process which develops over a period of time and which has differ-

ent phases. Testing the excitability of the pupal nerves at varying times during the process of inflammation may give entirely different results. This is reflected by the wider variation in data obtained in teeth with pulpitis than that obtained from teeth with normal pulps. Unfortunately, the results obtained do not permit the use of their technique in the clinical diagnosis of pulpitis, but their results should be highly important to all individuals using the electric pulp test as a means of diagnosis.

The mechanics of the relationship of the teeth and periodontium has been discussed by authors in the fields of prosthesis and restorative dentistry. Smyd (p. 860) used as an approach a simplified experimental method to evaluate stress factors in the periodontium in natural teeth and in those supporting fixed partial prostheses. He demonstrated that fixing of prosthetic appliances to two teeth changes the characteristic relation of the individual teeth and places the center of rotation in the nontooth-bearing bone between the abutments. Such an approach to the problem permits evaluation of individual factors.

A paper on "Musical Therapeutics" (p. 871) may appear as unusual in this JOURNAL. The author made an effort to evaluate the subjective responses of the patients and presents the most scientific approach to this subject. Unfortunately, patients' apprehensions and pains cannot be measured in millimeters, grams, seconds, or degrees.

Reprints of the Biographical Directory of Members published in the October issue are available from Dr. E. H. Hatton, 311 E. Chicago Ave., Chicago, Ill., for $1.00 per copy.

A very interesting report of the results of a survey supported by a grant of $20,000 from the Markle Foundation to the National Research Council has come to our attention. The report, which appears in the Annual Report of the Markle Foundation (1951) on pages 40 and 41, is as follows:

"National Research Council, Washington, D. C. Milton C. Winternitz, M.D., Chairman, Division of Medical Sciences, Grant: $20,000, 1951.

"Dental Research, in comomn with dental education and practice, has long been handicapped by its relative isolation from the broader fields of clinical medicine and the medical sciences. These limitations have been apparent in the functioning of the Committee on Dentistry, established in 1947 as an advisory body to the Armed Forces and the Veterans Administration. Expansion of the resources available for support of dental research in recent years has presented an opportunity to pierce these barriers and bind the study of oral biology and medicine into a more organic relationship than heretofore with that of the body as a whole, in keeping with the inter-disciplinary approach which is a cardinal principle of the Council's activities.

"The present grant has been used to survey the field and determine how the Committee might best be organized and employed to serve as a catalytic agent in this process. In the Spring of 1951, two ad hoc conferences were held to explore the possibilities. Dr. A. LeRoy Johnson, who launched the Harvard School of Dental Medicine on its pioneering course, joined the resident staff for three months to develop the program, and has since served as a consultant. In July, the Committee was reorganized to provide a broader base in the fundamental sciences; while dental members still predominate, such disciplines as biochemistry, pharmacology, endocrinology, microbiology, genetics and biometrics are also represented in an integrated structure. This reorganization has already been productive in the routine activities of the group, and gives promise of fulfilling its wider purposes through encouragement of fundamental government programs and stimulation of interest in oral medicine on the part of scientists in other fields. Other possible activities, such as symposia on basic approaches to dental problems, are under discussion at the present time.

"But the cut-and-try period is not over. Scientific personnel in the dental field are comparatively few. The doubtful value of many of the research projects submitted to the Committee reflect the limitations imposed by conventional academic and professional prerogatives. While this situation makes the going slow, it does give special meaning to the Committee. Changes in membership are progressively toward men of known ability and interest, whether dentist, physician, or experimental scientist. Not only has this trend enhanced the value of the Committee to the Armed Forces, but also its influence extends, via its members, to the schools to which they are attached."

This, of course, is a scientific survey and the investigators are entitled to their opinions.

H. B. G. R.

A TRACER STUDY OF THE TRANSFER OF F^{18} TO TEETH BY TOPICAL APPLICATION

HOWARD M. MYERS, D.D.S., JOSEPH G. HAMILTON, M.D., AND HERMANN BECKS, M.D., D.D.S.

From the Division of Dental Medicine, College of Dentistry, The George Williams Hooper Foundation for Medical Research and the Divisions of Experimental Medicine, Radiology, Medical Physics and Crocker Radiation Laboratory, University of California, San Francisco and Berkeley, Calif.

THE success of topically applied 2 per cent NaF solutions as a caries preventive agent[4, 5, 9] has been tacitly assumed to be related to the uptake of fluoride ions by sound enamel.[12] Accordingly, a number of attempts, to measure, by chemical means, the uptake of fluorides by enamel, have been made.

Norvold, Inglis, and Armstrong,[8] were able to demonstrate an increased percentage of fluoride ions in extracted teeth which were immersed in a 0.2 per cent solution for intervals of 100 to 200 minutes. However, Armstrong and Knutson,[2] using teeth of children receiving 2 to 6 clinical applications of a 2.0 per cent solution, found by chemical analysis no increase in the fluoride content of the enamel. They suggested that any increase that might be found would be located in the surface layer of enamel and that it would be of a magnitude small enough to be obscured by measurements performed on the whole enamel. The outer 10 per cent of enamel was analyzed by Syrrist[11] who exposed children's teeth to 2 and 4 per cent NaF solutions for 20 to 40 minutes. It was found that when pooled samples of 10 treated teeth were analyzed and compared to similar samples of untreated teeth, a definite increase in fluorine content was observed.

To avoid the problem of analyzing the teeth for fluorine, Straub and Adler[10] performed an in vitro study on fragments of enamel in which the solution containing the NaF was analyzed before and after varying lengths of contact with the enamel. An equilibrium between the fluorides of enamel and solution was established in 1 hour at which time the enamel had increased in fluoride content by 3.6 times. Adler, Straub, and Popovies[1] used the same method of analyzing the solution in an in vivo study on human teeth. They were able to show marked decreases in the fluoride content of the solution bathing the teeth when variations in technique were adopted.

All of these investigations have had as their goal the determination of whether or not quantitative amounts of fluorine are transferrable to teeth. Such information would be of aid in furthering our understanding of the mechanisms of action of fluoride ions applied in this manner. The chief difficulty in obtaining such data by chemical means lies in the fact that there is no way to distinguish between the fluoride ions already present in a tooth and the small amount added to the enamel by the experimental procedure. The use of radioactive fluorine offers the possibility of circumventing this problem. In addition,

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the radioautographic technique can be expected to yield information about the site of fluorine deposition in teeth. All of the radioactive isotopes of fluorine, however, have short half lives and only one, F^{18}, can be regarded as a potentially useful aid in tracer studies.

METHOD AND MATERIAL

Carrier free F^{18} was produced in the 60 inch cyclotron of Crocker Radiation Laboratory in Berkeley, California, by bombarding the O^{16} of triple distilled water with alpha particles. The reaction may be indicated as follows:

$$_8O^{16} + {}_2He^4 \rightarrow {}_9F^{18} + {}_0n^1 + {}_1H^1$$

F^{18} decays by positron emission with a half life of 112 minutes to form $_8O^{18}$, a stable isotope of oxygen.

Upon completion of the bombardment the water containing the F^{18} was made slightly alkaline to prevent the loss of HF^{18} and 20 mg. of NaF were added. The solution was then evaporated down to 1 ml. to obtain a 2 per cent NaF solution containing radioactive F^{18}.

Advantage was taken of the positron decay of F^{18} [3] to facilitate analysis. Positrons, immediately upon being emitted, collide with nearby electrons and disappear, leaving 2 gamma photons in their place.[6] These photons were used for counting purposes inasmuch as no correction for self absorption would be necessary. A filter was interposed between the sample and counter to absorb all positrons prior to their reaching the sensitive volume of the counter. In this way only the annihilation radiation was counted for all samples. Counting was accomplished with a Tracerlab Autoscaler and either a scintillation counter containing a thallium activated sodium iodide crystal or bell-shaped Geiger-Mueller tube with a window thickness of 4 mg./cm.^{2} The amount of F^{18} found was compared to a standard prepared for each bombardment and the results were determined as percentage of dose. In addition, the standard was used to plot a decay curve of the radioactive sample. This provided a measure of the purity of F^{18}. These curves were plotted for more than 10 half lives and no evidence of the presence of any long lived contaminants (14.8 hour Na^{24} from the glass cell) was found.

Teeth from patients varying in age from 13 to 70 years were selected for study. Only specimens which were not pulpless and were free from caries as indicated by roentgenograms and clinical examination were chosen. The specimens were divided into 2 main groups: one treated in situ and the other treated after extraction. The latter group was further subdivided to furnish samples for radioautographic studies.

The in situ teeth were pumiced lightly to remove the soft deposits. They were then isolated with a rubber dam or cotton rolls and dried with compressed air. One-tenth milliliter of radioactive solution was applied with a cotton pledget held in a cotton pliers to the exposed surfaces of each tooth for a period of ½ minute. The tooth was allowed to dry in air for 5 minutes after which the patient rinsed his mouth 4 times. The tooth was extracted ½ hour after treatment, analyzed along with the pledget and mouth rinses, and then coated

with sticky wax.* Eight milliliters of 10 per cent HCl was used to bathe the tooth for 45 seconds and the acid was then analyzed to determine the percentage of dose found in the dissolved layer of enamel. This procedure was repeated until no F^{18} was detectable in either the acid bath or the tooth. The mouth rinses were evaporated to 8 ml. and analyzed directly. Pledgets were teased apart and diluted in a large volume of water. An aliquot of this was taken for radioassay.

Studies of the uptake of F^{18} were also made on teeth which had been extracted some time previously. These specimens were kept in physiologic saline until ready for use. They were then pumiced and the roots were covered with wax in the manner described above, to assure that only the enamel was exposed to the solution. Topical applications were made with cotton pledgets and the teeth were allowed to dry in air. Rinsing was accomplished by passing 8 ml. of water from a serological syringe over the crown. Half of these specimens were analyzed quantitatively for activity and the other half were embedded in plastic for a radioautographic survey.[7] Sections of the teeth used for obtaining radioautographs were studied with Grenz rays, infrared and ultraviolet photography to illustrate the surface condition of the enamel.

FINDINGS

The percentage of recovered dose of F^{18} obtained in intact teeth, acid layer, pledgets and rinses in the teeth studied in situ is presented in Table I. It can

TABLE I

PER CENT OF RECOVERED DOSE FOUND IN IN VIVO TEETH

LOCATION	AGE	WHOLE TOOTH	LAYER 1	LAYER 2	PLEDGET	RINSE 1	RINSE 2	RINSE 3	RINSE 4
$\overline{\|3}$	47	0.17	0.094	bg	97	1.8	0.37	0.23	0.10
$\overline{\|2}$	47	0.14	—	—	98	1.5	0.29	0.18	0.081
$\overline{\|1}$	47	0.28	0.24	bg	96	3.0	0.59	0.37	0.16
$\overline{1\|}$	47	0.31	0.21	bg	95	3.4	0.67	0.42	0.19
$\overline{2\|}$	47	0.11	—	—	98	1.2	0.24	0.15	0.065
$\overline{\overline{4}\|}$	13	0.069	0.057	bg	99	0.57	0.25	bg	0.030
$\overline{4\|}$	13	0.074	0.061	bg	98	1.3	0.46	0.27	0.090
$\overline{6\|}$	70	0.32	0.27	0.074	97	1.4	0.98	0.38	0.19
$\overline{4\|}$	13	0.065	0.053	0.015	100.0	0.28	0.056	0.0018	0.0018
$\overline{\overline{4}\|}$	13	0.10	0.076	0.027	100.0	0.24	0.049	0.0018	0.0018
Mean		0.16	0.13	0.039	98	1.5	0.39	0.19	0.091
SD		±0.097	±0.084	±0.025	±1.5	±1.05	±0.29	±0.16	±0.071
SE		0.032	0.032	0.018	0.50	0.37	0.10	0.059	0.025

be seen that an average of 0.16 ± 0.097 per cent of all the detectable radiation recovered was present in the intact teeth. From these data it may be calculated that the mean weight of fluoride ions present in one tooth after a single topical application and rinsing is 1.5 micrograms. The range within which 67 per cent of the scores fall is 0.60 to 2.3 micrograms. Approximately 80 per cent of the activity found in the intact tooth is present in the first layer of enamel. The

*The edge of the wax followed the shape of the cemento-enamel junction and lay on the enamel surface a distance of 2 mm. from the cervical line of the tooth. In this way only the root and a small portion of the crown were covered with wax.

second layer of enamel showed detectable amounts of F^{18} only in a few instances. The symbol "bg" indicates that the radioactivity of the sample was indistinguishable from the background of the counter.

The data derived from the pledgets indicate that 97 ± 1.5 per cent of the recoverable activity never leaves the cotton pledget during the application of the solution.

It is noteworthy that the amount of F^{18} removed by the first mouth rinse is almost 10 times the amount retained by the tooth. Successive rinses show decreasing amounts of activity. An estimate of the amount of fluorides present on the tooth before rinsing may be obtained by adding the mean values of the tooth and rinse data. The calculated result is 2.3 per cent of the recovered dose. This means that about 90 per cent of the fluorides transferred to the tooth during topical application is removed by rinsing.

Table II contains the data gathered from teeth treated in vitro. Only one rinse was made because the degree of similarity between the washing procedure used and actual rinsing of the mouth could not be evaluated. Teeth treated after extraction appear to have a greater uptake of F^{18} than those treated in situ. However, since the Critical Ratio between the former and the specimens in Table I is less than 2.0, no statistically significant difference can be established. The pattern of distribution of F^{18} observed for the in situ teeth, pledgets and rinses, is roughly the same as in Table II.

TABLE II

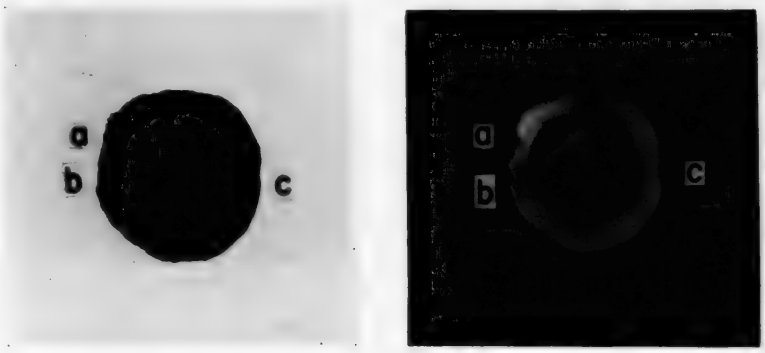
PER CENT OF RECOVERED DOSE FOUND IN IN VITRO TEETH

LOCATION	WHOLE TOOTH	PLEDGET	RINSE
$\frac{5}{}$ |	1.8	98	1.6
| $\frac{6}{}$	3.2	90	7.0
$\frac{7}{}$ |	1.7	95	3.6
$\frac{4}{}$ |	1.6	96	2.0
| $\frac{4}{}$	1.6	98	0.44
| $\frac{5}{}$	0.70	99	0.59
Mean	1.5	96	2.5
SD	±2.0	±3.0	±2.2
SE	0.90	1.3	1.0

Representative radioautographs obtained from teeth treated with F^{18} are shown in Figs. 1 to 4. These are combination radioautograph-roentgenograms which show blackening produced by F^{18} superimposed on light roentgenograms of the same sections. Each figure also illustrates the imperfections of the surface enamel of the same section. The teeth represented in Figs. 1 and 2 were rinsed as described earlier while those of Figs. 3 and 4 were not rinsed after drying the tooth.

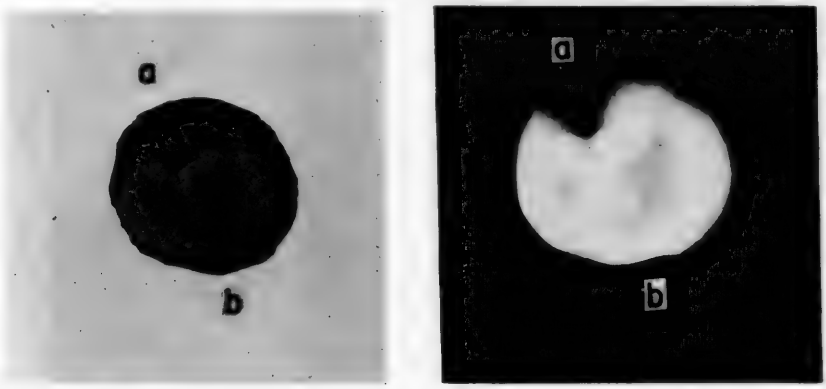
In Fig. 1, *A*, at *a*, *b*, and *c*, 3 dark, black areas can be seen. These correspond in location to the areas similarly marked in part *B* of the figure. The grey zone surrounding the periphery of the enamel in part *A* of Figs. 1 and 2 is a portion of the background darkening due to the roentgen rays, the remainder of the background having been masked out. The three zones where F^{18} has

accumulated appear macroscopically as areas of white and brown discolorations. In part *B* of Fig. 1 Grenz rays were employed to demonstrate the presence of radiolucency of the enamel at these sites.



A. *B.*

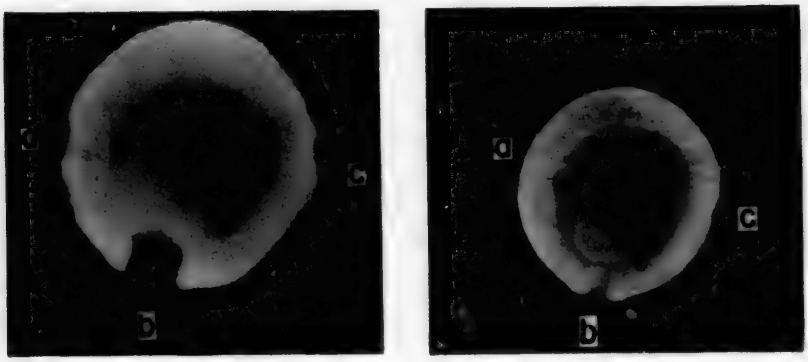
Fig. 1.—*A*, Radioautograph-roentgenogram of a human premolar tooth treated in vitro with fluorine and washed with 8 ml. of water. Concentration of F^{18} may be seen at *a*, *b*, and *c*. Activity of section: Approximately 1 microcurie; Thickness: 0.3 mm. Film: Industrial Type A X-ray film. Exposure time. 12 hours. *B*, Roentgenogram of section in part *A* showing surface imperfections at *a*, *b*, and *c*.



A. *B.*

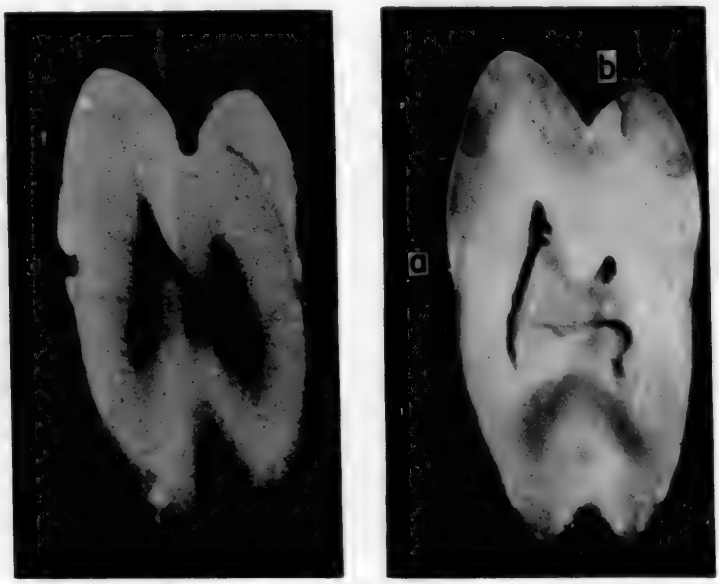
Fig. 2.—*A*, Radioautograph-roentgenogram of a human premolar treated in vitro with F^{18} and then washed with 8 ml. of water. Concentration of F^{18} may be seen at *a* and *b*. Activity of section: Approximately 1 microcurie; Thickness; 1.4 mm. Film: Industrial Type A X-ray film. Exposure time: 12 hours. *B*, Roentgenograph of section seen in part *A* showing surface lesions at *a* and *b*.

Fig. 2 presents a tooth with 2 main zones of F^{18} uptake. A carious lesion is present at *a* and F^{18} may be identified in the enamel in amounts sufficient to cause a blackening of the film. The blackening in the region of the dentino-enamel junction is apparently due to penetration by the x-rays since it also appears in the roentgenogram (Fig. 2, *B*). A surface imperfection at *b* is the site of another concentration of radioactive fluorine where the enamel surface showed a brown stained depression.



A. *B.*

Fig. 3.—*A*, Radioautograph-roentgenogram of human premolar tooth treated topically with F^{18} under in vitro conditions. Tooth not rinsed after application. General surface uptake of F^{18} may be seen as well as concentration at specific sites at *a*, *b*, and *c*. Activity of section: 1 microcurie (Approximately); Thickness 0.9 mm. Film: No-Screen X-ray film. Exposure time: 12 hours. *B*, Ultraviolet photograph of section showing the appearance of the sites of localization of F^{18}. Points *b* and *c* are easily visible while *a* shows only a faint whitish area.



A. *B.*

Fig. 4.—*A*, Radioautograph-roentgenograph of a human premolar painted with F^{18} in vitro and not washed prior to exposure to film. F^{18} can be seen over the entire crown and at the site labelled *a*. Activity of section: Approximately 1 microcurie; Thickness: 0.9 mm. Film: No-Screen X-ray film. Exposure time: 12 hours. *B*, An infrared photograph of section made to illustrate chalky white area at gingival third of buccal surface (*a*). Crack running through lingual cusp is artifact due to drying out of section after its exposure to film.

A cross section of another tooth treated after extraction is seen in Fig. 3. Here the grey background from the roentgen rays is seen in its entirety. The effect of the F^{18} can be recognized by its dark black appearance. The entire periphery of the unwashed enamel shows an uptake of F^{18}. Three main points of accumulation and penetration may be seen at *a*, *b* and *c*. The surface conditions at these sites are shown by an ultraviolet photograph in part *B*. Obvious defects are seen at *b* and *c*, while only a faint white discoloration can be discerned at *a*.

Fig. 4, *A* demonstrates a longitudinal section through a premolar which exhibits general surface uptake on this unwashed tooth. The area at *a* appears macroscopically as a chalky white zone. This same area by infrared photography (Fig. 4, *B*) is seen to have a different appearance than normal enamel. Grenz rays did not disclose any difference in radiopacity of the enamel. The crack in the region of the lingual cusp at *b* is a result of drying of the section.

DISCUSSION

The radiochemical and radioautographic data suggest that the fluorides present on a tooth immediately after topical application can be divided into 2 categories. The first of these is the fluorides on the intact surface of the enamel which can be readily removed by rinsing with water. The second type is retained in the surface defects of the enamel and is not removed by washing. These fluoride ions can be seen to penetrate such areas in large amounts forming points of concentration in the enamel. It is suggested that the protective action of topical fluorides is related to its uptake by these imperfections rather than to the general surface inasmuch as the latter is so readily removed from the tooth.

Because the half life of F^{18} is so short, these findings should be regarded as indicative of what happens only during the hours immediately following the application. Changes which occur later than this cannot be studied with F^{18}. In addition, the role of multiple applications remains unexplored with this tool and must be evaluated by other methods of study.

CONCLUSIONS

1. By means of topical application, approximately 2 per cent of the fluoride ions on the cotton pledget are transferred to the tooth.

2. The major portion of the fluorides which are transferred to the tooth are removed by means of rinsing. This portion appears to come from the surface of the sound enamel.

3. The portion of the fluorides which is not removed by rinsing is concentrated in the surface defects and discolored enamel of the crown.

4. Some of the sites of fluorine concentration are radiolucent and may be demonstrated by roentgen or Grenz rays. However, other lesions do not exhibit this property and must be demonstrated by infrared or ultraviolet photography.

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THE EFFECT OF SODIUM FLUORIDE UPON THE SURFACE STRUCTURE OF POWDERED DENTAL ENAMEL

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ON THE basis of electron microscope and diffraction experiments, Gerould[1] concluded that calcium fluoride is formed on the surface of enamel slabs when immersed in concentrated solutions of sodium fluoride for one month. Following this treatment the ordinary etching techniques routinely employed to enhance variations in surface structure failed to produce any appreciable effects, thus indicating that the fluoride treatment had reduced the surface solubility of enamel. He also found that, when powdered enamel was treated for one month with a 4 per cent sodium fluoride solution, x-ray diffraction patterns indicated that the original apatite structure had been replaced to an extent of 80 to 90 per cent by that of calcium fluoride. Other investigators have likewise reported that the surface layers of dental enamel are transformed from apatite to calcium fluoride upon treatment with sodium fluoride solution. One group of investigators[2] reported, on the basis of electron diffraction experiments, that the original apatite pattern is replaced by that of calcium fluoride in as short a period as 3 minutes upon immersion in a neutral 2 per cent solution of sodium fluoride.

Any increased knowledge concerning the mechanism whereby fluorides reduce the decalcification of dental enamel surfaces is of obvious importance in the general problem of caries resistance. It was felt that an exhaustive study using experimental techniques of electron diffraction, x-ray diffraction and both electron and light microscopy, using both powdered enamel and whole tooth sections, and by the use of various fluoride reagents administered as constituents of the diet and as applied topically in aqueous solutions would be of value in adding to this area of knowledge.

In the present study, powdered dental enamel was treated with solutions of sodium fluoride and of hydrofluoric acid under various controlled conditions. Electron diffraction and x-ray diffraction patterns were taken of the samples before and after treatment.

PROCEDURE

In all cases the enamel used as a starting material was prepared by grinding to a fine powder of 300 mesh some enamel previously separated by a modification of the Manly-Hodge flotation procedure.[3] For each preparation about 100 mg. of this powdered enamel were placed in a 15 ml. Pyrex centrifuge tube, and 5 milliliters of the appropriate fluoride solution were added from a pipet. The tube was immediately placed in a mechanical shaker for the designated period of time. The tube was then removed and its contents filtered through paper.

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Fig. 1.—Electron diffraction patterns. *a*, Normal apatite pattern of untreated powdered dental enamel. *b*, Calcium fluoride pattern. *c*, Composite pattern: upper segment is enamel after an HF treatment; lower left segment is calcium fluoride; lower right segment is untreated enamel.

Redistilled fluoride-low water was placed in the tube, the tube shaken, and the contents poured through the same filter; this step served both to insure a quantitative transfer of all enamel particles to the filter paper and to provide some washing of the enamel. The filter paper and its contents were then permitted to air-dry.

Electron diffraction patterns were taken with the conventional electron diffraction set-up using an R. C. A. type E. M. U. electron microscope. Each specimen was mounted on parlodion-covered mounting grids which had been prepared by conventional procedures. Several methods of applying the powdered enamel to the grid were employed, but the simplest and at the same time the most consistent method involved merely touching the parlodion-covered grid to the sample. Considerable practice was necessary to obtain mounts on which the particles were neither so sparse that the parlodion pattern alone resulted, nor so populous that the mount was opaque to the electron beam. With practice it has become possible to prepare successful mounts almost every time. Each mount was checked in a preliminary way using the light microscope. The electron diffraction patterns were recorded photographically. The diameters of the rings on the pattern of each sample were measured with the aid of a Bausch and Lomb magnifying eye-piece with built-in scale. Relative intensities were estimated visually. Each pattern was identified by comparing its diameters and intensities with those of standard patterns of the starting enamel and of pure calcium fluoride. The standard patterns were also checked against data listed in the literature for these materials to verify that they were the correct standards. A pattern was identified as apatite or as calcium fluoride if it coincided fully with the standard. If both standards were required to account for the rings of the specimen, the pattern was identified as corresponding to both materials. In the latter cases, a visual estimate was made from the intensities of the strong lines from the two substances as to which material was predominate or whether they were of approximately the same intensity; these estimates must be interpreted as approximate.

X-ray diffraction patterns were taken on a Norelco diffraction unit, recording each pattern photographically. The specimen was mounted by coating a thin glass fiber very lightly with silicone grease and then rolling it in the powder to provide a coating of the sample on the film. The film and specimen were then inserted conventionally in the camera. Identification of each pattern was made by comparison with the standard patterns.

The data are summarized in Tables I through IV. In every case the identification stated in the Tables accounts completely for the pattern obtained. Typical electron diffraction patterns are shown in Fig. 1. A composite electron diffraction pattern is shown in Fig. 1, *c;* of the 3 segments, 1 consists of the CaF_2 standard, 1 of the apatite standard and 1 of a treated sample. In the composite, the treated sample is clearly that of calcium fluoride. It should be noted that the identifications were not made by means of these composite patterns, but rather by direct measurement on the original patterns. Although some detail is lost in reproduction, this figure is representative of the types of patterns obtained.

TABLE I

DATA ON SAMPLES AFTER TREATMENT WITH SODIUM FLUORIDE AT pH 3

CONCENTRATION	TIME OF TREATMENT IN MINUTES	ELECTRON DIFFRACTION PATTERN	X-RAY DIFFRACTION PATTERN
9050 γ F^-	60	CaF_2	
3000 γ F^-	60	CaF_2	CaF_2 + little apatite
3000 γ F^-	30	CaF_2	CaF_2 + little apatite
3000 γ F^-	15	CaF_2	CaF_2 + apatite
3000 γ F^-	5	CaF_2 + trace apatite	CaF_2 + apatite
1000 γ F^-	60	apatite + little CaF_2	
500 γ F^-	60	apatite + little CaF_2	
100 γ F^-	60	apatite	

TABLE II

DATA ON SAMPLES AFTER TREATMENT WITH SODIUM FLUORIDE AT pH 4

CONCENTRATION	TIME OF TREATMENT IN MINUTES	ELECTRON DIFFRACTION PATTERN	X-RAY DIFFRACTION PATTERN
9050 γ F^-	60	CaF_2	apatite
3000 γ F^-	60	CaF_2 + trace apatite	apatite + little CaF_2
3000 γ F^-	30	CaF_2 + little apatite	apatite
3000 γ F^-	15	CaF_2 + apatite	apatite
3000 γ F^-	5	CaF_2 + apatite	apatite
1000 γ F^-	60	apatite	
500 γ F^-	60	apatite	
100 γ F^-	60	apatite	

TABLE III

DATA ON SAMPLES AFTER TREATMENT WITH SODIUM FLUORIDE, pH NOT ADJUSTED

CONCENTRATION	TIME OF TREATMENT	ELECTRON DIFFRACTION PATTERN	X-RAY DIFFRACTION PATTERN
2050 γ F^-	24 hrs.	apatite	
300 γ F-	60 min.	apatite	apatite

TABLE IV

DATA ON SAMPLES AFTER TREATMENT WITH HYDROFLUORIC ACID

CONCENTRATION	TIME OF TREATMENT IN MINUTES	ELECTRON DIFFRACTION PATTERN	X-RAY DIFFRACTION PATTERN
19000 γ F^-	60	CaF_2	
3000 γ F^-	60	CaF_2	CaF_2 + little apatite

DISCUSSION

The data indicate that the treatments with sodium fluoride and with hydrofluoric acid do result in a direct transformation of the apatite into calcium fluoride. The data further indicate that the pH is of extreme importance in determining the rate of this transformation. There is no observed transformation even after 24 hours' treatment when the pH is around 6.5 to 7.0 (Table III), but the transformation goes readily at pH 4 even for the treatments with time intervals as short as 5 minutes, and even more readily at pH 3.

Electron diffraction patterns show up the change before x-ray patterns do, since the former is an indication of the localized surface structure and the latter of the more deepseated or overall structure of the particles of enamel. At pH 4, the change shows up after 5 minutes in a 3000 γ solution in the electron diffraction pattern and the pattern is almost entirely that of calcium fluoride after 1 hour of treatment. In the same series the change does not extend deep enough to show up in the x-ray pattern until the treatment is 1 hour, and then it shows up only to a slight extent. At pH 3, both types of pattern reveal that the transformation takes place much more readily than at the higher pH. With the lower concentrations of reagent, the change to calcium fluoride does not show up at all at pH 4, while it does to a slight but definite extent at pH 3. The hydrofluoric acid treatments are seen to be analogous to the low pH sodium fluoride treatments, as properly should be expected on the basis of the sodium fluoride results. These data include samples at varying degrees of conversion from apatite to calcium fluoride, and no intermediate crystalline compounds have been observed.

Additional experiments are underway in accordance with the general program outlined above. Although no definite conclusions can be reported as yet concerning the action of other fluorides as the reagent, other work now in progress indicates that the actions of the various reagents are not all similar.

SUMMARY

The treatment of the powdered enamel surfaces with sodium fluoride solution results in a direct transformation from an apatite to a calcium fluoride structure. The rate of this transformation is enhanced very markedly as the pH of the solution is lowered. The concentration of the fluoride solution and the time of treatment are also factors involved in the rate of the transformation.

The authors wish to acknowledge the very valuable technical assistance of Richard Scott, Patricia McGowan, and Kenneth McGowan.

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RELATIONSHIP BETWEEN pH, AGE, AND CONCENTRATION OF SOLUTIONS OF STANNOUS FLUORIDE AND SODIUM FLUORIDE IN DECREASING ENAMEL SOLUBILITY AND AFFECTING THE UPTAKE OF FLUORINE

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IN ALL instances in which stannous fluoride has been used in enamel solubility determinations[1, 3] or as fluoride supplements in in vivo experiments[4, 5] a marked precipitation of stannous hydoxide has occurred in a few hours after making the solutions. Thus it was of concern whether this precipitation is accompanied by a reduction in the ability to decrease enamel solubility.

Since stannous fluoride has been demonstrated to be superior to sodium fluoride in reducing the incidence of caries in rats,[4, 5] some knowledge of the reason for its superiority was of importance in considering the clinical value of stannous fluoride. One reason for the superiority of stannous fluoride may have been due to a greater uptake of fluoride by the tooth tissues with stannous fluoride than with sodium fluoride. Therefore, the purpose of the study reported here was twofold: (a) to investigate by in vitro enamel solubility techniques the effect of "aging" stannous fluoride solutions as compared with sodium fluoride and (b) to determine whether enamel takes up more fluorine from stannous fluoride than from sodium fluoride.

EXPERIMENTAL

The procedure for determining the solubility of powdered enamel was similar to that reported previously.[2, 3] In brief this was as follows: 5.0 ml. of either sodium fluoride or stannous fluoride was added to 100 mg. of powdered 100 mesh dental enamel. After shaking the mixture for 20 minutes, it was centrifuged and the clear supernatant solution discarded. After washing the enamel with distilled water, 10.0 ml. of 0.2 N acetic acid was added to the insoluble enamel, and the mixture again shaken for 20 minutes as before. Following prompt centrifugation the supernatant was decanted, made to a calculated volume with distilled water, and aliquots were analyzed for phosphorus. Controls were run for comparison except that fluorides were not added. Both sodium fluoride and stannous fluoride solutions were made fresh on day 1, and enamel solubility determinations were made immediately. The solutions were allowed to stand at room temperature for the designated periods of time.

The results of comparing the protective ability of sodium fluoride with stannous fluoride when each solubility determination was made on subsequent days is summarized in Table I. This table indicates the degree of protection effected by each fluoride solution when compared with controls in which no

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fluoride had been added. The data show that stannous fluoride solutions at a fluoride concentration of 100 p.p.m. quickly lose their marked effectiveness in reducing enamel solubility in vitro, but that the effect produced by sodium fluoride is not influenced by the age of the solutions. It is evident from Table I that after about 5 days there is no additional decrease in effectiveness of the stannous fluoride solutions, and after this time stannous fluoride remains unquestionably superior to sodium fluoride in its ability to reduce enamel solubility.

TABLE I

REDUCTION OF ENAMEL SOLUBILITY AFTER TREATMENT WITH SODIUM FLUORIDE OR STANNOUS FLOURIDE SOLUTIONS OF DIFFERENT AGES, AT A CONCENTRATION OF 100 MICROGRAMS OF FLOURINE PER MILLILITER

	REDUCTION IN ENAMEL SOLUBILITY	
DAY	STANNOUS FLUORIDE (%)	SODIUM FLUORIDE (%)
1	83	38
3	56	37
5	46	36
7	46	36
10	48	36
12	46	35

Since it was established that some "aging" effect takes place with stannous fluoride solutions at a fluoride concentration of 100 p.p.m., it was decided to study different fluoride levels and compare the enamel solubility-reducing effect with the uptake of fluoride by the enamel at each level, also as a function of age of the solutions. Again, sodium fluoride was run under a similar condition for comparative purposes. The method for determining fluorine in the enamel has been reported.[6] The procedure for determining enamel-fluoride uptake was essentially the following: after treating the enamel with the test fluoride solutions, the supernatant was decanted and the enamel washed with three, 1 ml. portions of fluoride-free water. To the enamel residue in the centrifuge tube, 10 milliliters of perchloric acid was added and

TABLE II

ABILITY OF DIFFERENT CONCENTRATIONS AND AGES OF SODIUM FLUORIDE AND STANNOUS FLUORIDE IN REDUCING ENAMEL SOLUBILITY AS COMPARED WITH THE AMOUNT OF FLUORIDE TAKEN UP BY THE ENAMEL

	50 μG F/ML.				100 μG F/ML.				500 μG F/ML.			
	SnF_2		NaF		SnF_2		NaF		SnF_2		NaF	
DAY	R.S.* (%)	F UPTAKE (P.P.M.)	R.S. (%)	F UPTAKE (P.P.M.)	R.S. (%)	F UPTAKE (P.P.M.)	R.S. (%)	F UPTAKE (P.P.M.)	R.S. (%)	F UPTAKE (P.P.M.)	R.S. (%)	F UPTAKE (P.P.M.)
1	76	27.6	30	12.6	80	40.6	31	17.2	98	85.6	44	28.0
3	34	23.2	30	7.9	47	42.0	31	16.2	98	78.1	43	24.4
5	31	20.7	29	9.1	46	44.2	34	11.8	92	76.6	42	25.8
7	35	41.4	33	7.6	48	63.7	31	10.6	93	106.6	44	12.1
8	34	34.1	31	11.2	47	77.7	31	14.3	92	173.8	43	29.7
10	35	42.3	29	11.5	47	102.7	31	13.7	92	296.8	42	24.9
10	25†	29.8†	28†	18.7†	31†	52.4†	30†	23.8†	68†	90.4†	41†	36.1†

*R.S. = reduction in enamel solubility.

†All the test solutions were adjusted to pH 4.0 immediately adding them to the enamel.

TABLE III

ABILITY OF STANNOUS FLUORIDE AND SODIUM FLUORIDE SOLUTIONS AT DIFFERENT AGES AND FLUORIDE LEVELS TO REDUCE ENAMEL SOLUBILITY. THE FINAL pH OF ALL THE SOLUTIONS AND THE UPTAKE OF FLUORIDE BY THE ENAMEL ARE ALSO SHOWN

	50 μG F/ML.						100 μG F/ML.						300 μG F/ML.						500 μG F/ML.					
	SnF_2			NaF			SnF_2			NaF			SnF_2			NaF			SnF_2			NaF		
DAY	R.S. (%)	F UPTAKE (P.P.M.)	pH	R.S. (%)	F UPTAKE (P.P.M.)	pH	R.S. (%)	F UPTAKE (P.P.M.)	pH	R.S. (%)	F UPTAKE (P.P.M.)	pH	R.S. (%)	F UPTAKE (P.P.M.)	pH	R.S. (%)	F UPTAKE (P.P.M.)	pH	R.S. (%)	F UPTAKE (P.P.M.)	pH	R.S. (%)	F UPTAKE (P.P.M.)	pH
1	75	18.0	3.7	30	8.2	5.8	79	30.7	3.6	32	11.2	6.0	89	51.0	3.4	34	13.7	6.3	96	68.3	3.3	39	20.5	6.3
3	65	33.7	3.3	30	10.6	5.5	74	60.3	3.1	30	12.7	5.7	88	82.8	3.0	31	16.6	6.0	90	122.5	3.0	32	23.4	6.3
5	49	40.3	3.0	27	10.0	5.5	49	88.9	2.9	28	12.4	5.6	84	122.8	2.8	29	18.3	5.6	91	185.3	2.7	37	23.8	6.2
8	37	38.2	3.0	24	7.9	5.4	46	97.0	2.9	27	11.2	5.4	83	211.0	2.7	28	15.3	5.6	90	304.0	2.6	34	19.9	6.2
10	37	43.0	3.0	20	7.2	5.6	44	95.2	2.8	23	11.2	5.9	80	198.4	2.6	30	17.7	5.7	90	374.0	2.4	32	24.0	6.3
13	35	36.0	3.0	20	--	5.4	42	78.7	2.8	23	--	5.7	79	196.9	2.6	31	--	5.8	84	355.6	2.4	30	--	6.3

the entire contents transferred to the distilling unit. From this point on, the procedure was similar to that outlined previously.[6] In order to correlate fluoride uptake and enamel solubility, 2 different enamel samples for each series of determinations were used, one of which was used for fluorine analysis while the other was used for the determination of enamel solubility.

Table II summarizes these data. It is evident that at a fluoride level of 50 and 100 p.p.m., stannous fluoride solutions show a decreasing effectiveness with age in protecting powdered enamel against solubility in weak acid, but not at a fluoride level of 500 p.p.m. Results similar to those presented in Table I relative to sodium fluoride were again found; thus it is evident that age has little influence on any concentration of this salt in affecting its ability to reduce enamel solubility. These more extensive data corroborate the evidence[2, 4] that stannous fluoride is superior to sodium fluoride in reducing enamel solubility. It should be noted that little additional effect on solubility occurs when the level of sodium fluoride is increased from 50 to 500 p.p.m. fluorine, but that quite a marked increase in protectiveness parallels the increase in fluoride concentration when stannous fluoride is used.

The data relative to the uptake of fluorine by the enamel when treated with sodium or stannous fluoride at different concentrations are given in Table II. At each particular fluoride level the uptake was considerably less in the case of sodium fluoride than with stannous fluoride. Also, the level continued to increase steadily with age in the case of stannous fluoride, but not with sodium fluoride. Further, as the added fluoride level of stannous fluoride increased from 50 to 500 micrograms, there was a considerable increase in fluoride uptake, while relatively little increase in fluoride uptake occurred in the case of sodium fluoride.

It is puzzling that there was an increase in fluoride uptake as the stannous fluoride solution was allowed to "age." One fact of significance is that there was a change in pH proportional to the amount of stannous hydroxide precipitated. Thus the pH appeared to change as a function of time. To test this, all the solutions were adjusted to pH 4.0 at the end of day 10, and the entire run for day 10 was repeated. Line 8 in Table II indicates the results of this treatment. With stannous fluoride both the uptake of fluoride and the protective effect were reduced, whereas with sodium fluoride the uptake was increased and the protective effect remained unchanged. No explanation is offered as an interpretation of this finding, although it may involve a precipitation of fluorine as calcium fluoride or even as fluorapatite.

Because so much stannous hydroxide had been removed from the solution after hydrolysis had gone on for several days, it was reasonable to assume that the solutions were not comparable to those used at the beginning of the experiment. Therefore, an additional experiment was planned and the data are presented in Table III. In this table, the level of 300 p.p.m. fluorine as sodium and stannous fluoride was included as well as the change in pH of the various solutions as a function of time.

At each fluoride level used, stannous fluoride was definitely superior to sodium fluoride in reducing enamel solubility. The "aging" effect of stan-

nous fluoride was evident at the 50 and 100 microgram fluorine level, but when the level reached 300 micrograms or above little aging effect was noted. This corroborates the data reported in Table II. Sodium fluoride, regardless of the fluoride level or the age of the solution, was not affected.

These results give additional indication that stannous fluoride may be superior to sodium fluoride in preventing human dental caries, especially when the method of topical application is used. Moreover, the superiority of freshly prepared solutions of stannous fluoride is demonstrated. It is indicated that the superiority may be related to the greater uptake of fluorine.

SUMMARY

The marked effectiveness of freshly prepared stannous fluoride solutions in reducing the solubility of powdered dental enamel decreases over a period of a few days, but the effectiveness of sodium fluoride solutions is not decreased. Enamel takes up larger amounts of fluorine from stannous fluoride regardless of the age of the solutions, and the uptake is greater from old stannous fluoride solutions than from those which are freshly prepared. The uptake of fluorine from sodium fluoride is not affected by age. These changes appear to be related to the pH of the solutions. The pH of the stannous compound decreases, through hydrolysis of the salt, but the pH of the sodium salt remains unchanged. Aged dilute solutions are decidedly less effective than those freshly prepared in reducing the solubility of dental enamel.

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THE FORMATION OF HYDROXYFLUOR-APATITE IN TOOTH ENAMEL UNDER INFLUENCE OF DRINKING WATER CONTAINING FLUORIDE

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THE question of why drinking water containing fluoride should effect a reduction of dental caries remains unanswered. The fact that fluoride is a fermentative poison has led to the presumption that a fermentative mechanism in the tooth may be damaged, or that a bacteriostatic effect may be achieved. Experiments with rats have shown that dental caries is inhibited by feeding with large doses of sodium fluoride (250 mg. per kilogram of diet) and of monoiodoacetic acid (200 mg. per kilogram of diet), which is also a fermentative poison.[1] This explanation, however, does not seem to be applicable to the small fluoride concentrations found in drinking water, since in these no fermentative damage or bacteriostatic effect will take place.[2, 3] A similar explanation of the inhibitory effect of fluoride, which also seems scarcely defensible, is that dentin phosphatase is prevented from dissociating phosphate ions, by which in an unexplained way the resistance to dental caries, and also mottled enamel, are said to be caused.[4] By further experiments with animals, however, it was proved that the phosphatase activity in plasm, in bones, and in the teeth is not changed even by feeding large doses of fluorides.[5]

Although none of the known ferments will be inhibited by the small fluoride concentrations in drinking water, there is the possibility of a disturbance of vital functions. This direction is indicated by an observation[6] that under influence of fluoride the ameloblasts will degenerate, and that mottling of enamel may result. Since, moreover, an antagonism between iodide and fluoride is assumed to exist in the hormone production of the thyroid gland,[8] and as only the hormone of the thyroid gland containing iodine possesses a sympathicotonous effect, the inhibitory effect of fluoride may possibly be explained by the fact that the sympathicotony of the organism, and particularly of the salivary glands, is reduced,* so that a larger quantity of more liquid saliva is produced, and that the resistance to dental caries is thus increased.[11]

Enamel resistant to dental caries contains more fluoride than enamel susceptible to dental caries.[12, 16] The fact that it is doubtlessly possible to reduce dental caries by painting enamel with a solution of sodium fluoride points to the local effect of fluoride on enamel. Various explanations of this fact have been given: thus the fluoride in the enamel is said to effect an "active attraction of calcium from the saliva by which the spaces between the prisms of the enamel are barred to the infiltration of bacteria."[17] Moreover, it has been said

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*For remarks on the endocrinologous effect of fluoride see Hartmann, Chytrek, and Ammon[9] and Fiorentini.[10]

that the activity of enolase and phosphatase is reduced by fluoride in enamel, and that the resistance to dental caries is thus increased in an unexplained way.[18, 19] Finally, it was assumed that fluoride "contributes to a more rapid and more complete maturation of the enamel as a kind of catalyzer" and that "with the help of dissociated ions of calcium and rhodanum an organoapatite is composed to a submicroscopic, morphologically functioning unity in the enamel."[20] On the other hand, the radiographic and electron microscopic illustrations of Gerould[21] show that enamel containing fluoride and which has developed under influence of drinking water containing fluoride will possess a pure apatite structure. From this, Gerould concluded that enamel of this kind contains fluor-apatite, which in subtle distribution is disseminated through the hydroxyapatite and the carbonate-apatite* of the enamel. Furthermore, Gerould assumes that this enamel-containing fluoride is distinguished from hydroxyapatite by being less easily soluble, and thus possesses an increased resistance against acid. Gerould also attempts to explain the increased caries resistance of enamel-containing fluoride by the fact that under influence of acid a soluble fluoride of bacteriostatic effect will be freed, but it is hardly possible to use the latter assumption in explaining the inhibitory effect of fluoride, as enamel rich in fluoride (mottled enamel) does not contain more than about 0.035 per cent of fluoride.[23]

Pure fluor-apatite is *not* stable, but only isomorphous mixtures of hydroxy-apatite (=HA) and fluor-apatite (=FA), which are called hydroxyfluor-apatite (=HFA) *are* stable in the presence of water. Therefore, it may contribute to the knowledge of the inhibitory effect of the fluorine ions, if experiments on the production of this isomorphous HFA in a pure form and on ascertaining its solubility[22] are considered. A description of experiments on the indication of the solubility of HA and HFA shall be given. In view of the results of these experiments, the formation of HFA in enamel under influence of drinking water containing fluoride and the possibility of the inhibitory effect in this formation of HFA in the enamel shall be discussed.

PRODUCTION OF HA-FA MIXTURES BY ACIDIMETRIC PRECIPITATION

Solutions of calcium chloride and phosphatic acid were slowly dropped from 2 burettes into 2 liters of boiling water, which had been instilled with an indicator of an almost neutral change-point, e.g., bromthymol blue. By dropping in sodium hydroxide from a third burette the reaction was kept permanently neutral to the indicator. After a short time a sediment of pure HA originated with increasing opacity, as may be gathered from the amount of sodium hydroxide that had to be added to achieve permanent neutrality:

$$(1)\quad 10\ CaCl_2 + 6H_3PO_4 + 20\ NaOH \rightarrow \underbrace{3\ Ca_3(PO_4)_2 \cdot Ca(OH)_2}_{\text{Hydroxyapatite}} + 20\ NaCl + 18\ H_2O^*$$

When, in another experiment, various amounts of a sodium fluoride solution were dropped in from a fourth burette simultaneously with the other three solutions, a smaller quantity of sodium hydroxide was needed to achieve

*As shown before,[22] a combination of carbonate-apatite, however, is not able to exist in the presence of water, and therefore it is not contained in dental tissues.

permanent neutrality. Also, this finding can be demonstrated by the equation:

(2) $10\ CaCl_2 + 6\ H_3PO_4 + 2\ NaF + 18\ NaOH \rightarrow 3\ Ca_3(PO_4)_2 \cdot CaF_2 + 20\ NaCl + 18\ H_2O$*
Fluor-apatite

Formula (2) shows that the quantity of sodium hydroxide needed to produce pure FA is, theoretically, 10 per cent smaller (18 instead of 20) than the amount required to produce HA according to equation (1). When, however, various amounts of sodium fluoride solutions were added, there was never a consumption of sodium hydroxide 10 per cent smaller, but at the most a consumption 7 per cent smaller than without addition of sodium fluoride. From this it was concluded that pure FA cannot exist in the presence of water, but that only mixtures of HA and FA, which contain no more than 70 per cent of FA, will originate.

As HA and HA-FA mixtures have low speeds of crystallization and thus will crystallize slowly from a watery solution, it is important that the precipitation should be carried out slowly enough. Otherwise compounds which crystallize more rapidly and are more soluble would be formed, e.g., dicalcium phosphate† ($CaHPO_4 \cdot 2\ H_2O$) and calcium fluoride (CaF_2). Thus several weeks are necessary to crystallize a few milligrams of apatite from some liters of water at 37° C., while at boiling temperature the speed of crystallization is so much increased that under the conditions described about 1 to 2 milligrams of apatite can be produced every minute.

INDICATION OF THE SOLUBILITY OF THE HA-FA MIXTURES

After the method of acidimetric precipitation described above, mixtures of HA and FA containing different amounts of FA were produced and suspended after filtrations and washing out distilled water free of carbonic acid. Having been shaken for different periods of time, the suspensions were filtrated, and the content of calcium oxide and phosphoric acid in the product of filtration was measured. It was found that the content had become constant after being shaken for four hours, so that the beginning of solubility balances was assumed.

TABLE I. SOLUBILITY BALANCES OF HA-FA MIXTURES IN WATER

PERCENTAGE OF FLUOR-APATITE	CaO (MG./L.)	P_2O_5 (MG./L.)	F' (γ/L.)
0 (pure HA)	3	2	0
5	1	1	2
10	1	0.5	3
20	0.5	0.2	3
35	0.5	0.3	6
70	0.5	0.3	10

From Table I it can be determined that the solubility of the HA-FA mixtures will be considerably diminished by a small content of FA, and that there is only a slight difference between mixtures of higher FA contents. In conse-

*The equations have to be composed such as to keep the right sides free of acid or alkaline substances besides of difficultly soluble compounds, as the reaction was kept permanently neutral to an indicator.

†When dicalcium phosphate originates during the acidimetric precipitation, e.g., when the speed of titration is too high, a consumption of sodium hydroxide smaller than that in the production of HA is found, so that the consumption of sodium hydroxide is a simple and subtle indication of the purity of HA.

quence to this decrease of solubility (as compared to pure HA), the HA-FA mixtures are isomorphous.*

DISCUSSION

Importance of the Duration of the Influence of Drinking Water Containing Fluoride.—According to the general tendency for compounds to be naturally as insoluble as possible, the HFA mixtures, being much less soluble than pure HA, by contact with drinking water containing fluoride HA is converted into HFA (HFA containing less fluoride is converted into HFA richer in fluoride). For this purpose, the influence of drinking water containing fluoride has to be long due to the low speed of crystallization of the apatites. After a stay of two to three years in regions with drinking water rich in fluoride, children show caries resistance equal to that shown by children living permanently at those places.† Experiments on the addition of sodium fluoride to drinking water provide a cue as to the duration of fluoride influence necessary for reducing dental caries. In Newburgh the caries susceptibility of the children was reduced after one year from 63.5 per cent to 55.2 per cent and, after another year, to 47.3 per cent.

Influence of the Concentration of Fluoride in Drinking Water.—The amount of fluoride contained in normal saliva (about 0.1 mg. per liter[26, 27]) and that contained in ordinary drinking water, are approximately at the same level. As shown in Table I, however, both these fluoride concentrations exceed the saturation concentration of the HFA mixtures (less than 10 γ per liter. This leads to the assumption that the HFA of the enamel probably contains little fluoride at first,‡ but is augmented gradually by FA. This assumption is supported by enamel analyses which show that the enamel of adults§ normally contains more fluoride than that of children.

As a result of direct contact with the enamel, and because of the possible increase of fluoride concentration in the saliva (by way of the body fluids) drinking waters rich in fluoride most probably will produce a larger and more rapid increase of fluoride in the enamel. As shown by many analyses,[13, 16] in regions with drinking water rich in fluoride, even the enamel of children will possess an increased content of fluoride and will, therefore, be less affected by caries than in regions with a low fluoride content in drinking water. The considerable decrease of solubility in the HFA mixtures, which is effected even by a small content of FA, and the small influence of a larger FA content on the

*Isomorphism is the capacity of two substances to build, when crystallized, from solutions, instead of separate crystals, one common crystal, which has different qualities, e.g., another solubility, than the separate crystals. The condition necessary for the origination of isomorphous mixed crystals (i.e., that the radii of the ions in the two substances have to be nearly identical) is present in the HA-FA mixtures: the ion of the hydroxylic has a radius of 1.32 AE, that of fluor, one of 1.33 AE.

†The fluorine ions can penetrate from drinking water directly into enamel which, as shown by Wannenmacher[24] is semipermeable to ions. This also explains the observation that, in regions with drinking water rich in fluoride, the upper incisors, which come into particularly close contact with the drinking water, prove to be particularly resistant to dental caries.[25]

‡Evans and Phillips[28] have shown that rats, as well as cattle, contain very little fluoride after their birth, and that animals have received nearly all the fluoride through the food. Glock, Lowater, and Murray[29] found that the fluoride contained in human bones increases by 0.02 per cent per year.

§The enamel of adults should therefore be more resistant to dental caries than that of children; because of the effects of other influences, however, it will be difficult to observe this phenomenon.

solubility of mixtures perhaps provides the explanation of the permanent influence of drinking water containing fluoride as well as why painting the enamel with a sodium fluoride solution produces an inhibitory effect on caries only on children (with enamel containing little fluoride) and not on adults (with enamel richer in fluoride).

The way in which fluoride comes into the enamel from the saliva can partly be traced with the help of radioactive fluoride. Volker, Sognnaes, and Bibby[30, 31] proved by animal experiences that radioactivity is shown in this saliva after injections with radioactive fluoride, but it was impossible to prove any radioactivity of the enamel because of the short half period of the radioactive fluorine. The direct transmission of the fluoride into the enamel from outside is indicated by the observation that the outer coat of the enamel contains more fluoride than the inner layers of the enamel.[30]

Supporting the assumption that HFA is present in the enamel, there is also the statement of Cox, Matuschak, Dixon, Dodds, and Walker,[32] as well as that of Gins[33] that resistance to dental caries can be developed in the enamel. The larger content of FA in the HFA mixtures of teeth resistant to dental caries is also indicated by the finding[34] that the fluoride content of these teeth is larger on the average than the fluoride content of teeth susceptible to dental caries. Moreover, the statement of Morgen, Dean, and McCallum[35] that caries resistance of teeth is considerably stronger than would be assumed from the small quantities of fluoride analyzed can be explained with the existence of HFA in the enamel, since, according to Table I, the solubility of the isomorphous HFA mixtures is considerably decreased even by a small FA content.

SUMMARY

According to a report on the production of pure isomorphous hydroxyfluorapatite (=HFA) by acidimetric precipitation from a watery solution, a description of experiments on the indication of the solubility of the HFA was given. It was found that the solubility of the HFA is considerably diminished even by a small fluorine content.

The probability that HFA may originate in the enamel by the influence of drinking water containing fluoride, and that the enamel should thus become less soluble and therefore be more resistant to dental caries is discussed.

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THE EFFECTS OF UREA AND SALTS OF AMMONIUM PHOSPHATE ON SALIVARY AMYLASE

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EARLY work established that 40 to 50 per cent urea effectively reduces the pH change[1] and the glycolytic activity[2] which occur in the dental plaque in the presence of glucose, possibly by denaturing the glycolytic enzymes.[2]

More recently, clinical use of low concentrations of urea (up to 13 per cent before dilution by oral fluids) and salts of ammonium phosphate (up to 5 per cent before dilution) for the control of dental caries has led to further investigation, particularly of the effects of low concentrations of these compounds on the oral structures and environment. The purpose of such studies has been to evaluate possible toxic side effects and to elucidate the mechanism by which the caries process is effected.

Thus, Lefkowitz and Tanchester[3] found an ammoniated dentifrice containing 13 per cent urea and 3 per cent dibasic ammonium phosphate to be without effect on the microscopic structure of the oral mucosa. Pearlman[4] and Pearlman and Hill[5] investigated the effects and mechanism of action of urea on *Lactobacillus acidophilus* and have concluded that, while with regard to acid production, concentrations of urea lower than 24 per cent (4 M) were ineffective, 1.5 to 6 per cent urea caused marked inhibition of growth, probably, as suggested earlier by Muntz and Miller,[2] by the mechanism of protein denaturation.

This report concerns an investigation of the effects of urea and salts of ammonium phosphate on salivary amylase in vitro, employing the concentrations of urea and ammonium phosphate which might be present in the oral cavity after the use of a "high urea" dentifrice. This investigation was undertaken for the following reasons: (1) it is of value to determine oral side effects of the use of urea; (2) the possible involvement of amylase in the dental caries process[6, 7] raises the question of whether an action of urea and ammonium phosphate on this enzyme may mediate the caries inhibitory action; and (3) the suggestion that urea affects glycolysis and growth of *Lactobacillus acidophilus* by means of a denaturing effect makes it necessary to investigate further the denaturing action of urea. Amylase is particularly suitable as a test enzyme, since it is readily obtainable, and the characteristics of its denaturation have previously been delineated.[8-12]

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Investigation of the relation of urea to denaturation* required that studies be made not only under physiologic conditions of temperature, pressure, and ion concentration, but also under conditions of elevated temperature and pressure and in the absence of salts. These conditions were introduced into the study because they modify the denaturation of amylase in known ways. Thus, high temperature[10, 13] or the absence of ions[8, 12] promotes denaturation, while high pressure slows or reverses one stage of the denaturation by counteracting the increase in the molecular volume of the enzyme associated with the reversible phase of the denaturation particularly.[13, 14]

METHOD

The salivary amylase used consisted of a powdered preparation prepared from 1 liter of mixed human saliva by alcohol precipitation. Before use in an experiment, a small quantity (usually 1 to 3 mg.) of this powder was added to 10 ml. of distilled water. This suspension was allowed to stand for 10 minutes, after which time it was filtered. The filtrate was dialyzed against distilled water for 24 to 48 hours at 4° C. and then was diluted, usually 1:40, with distilled water for use in an experiment.

The stock substrate solution was Merck soluble starch (according to Lintner), 1 Gm. per 100 ml., made up in distilled water according to the method suggested by Myers, Free, and Rosinski.[15]

Sodium chloride solutions were usually buffered with .004 M Na_2HPO_4-NaH_2PO_4. In those experiments or parts of experiments where NaCl and ammonium phosphate were lacking, no buffer was used. In all cases, however, the pH of the solutions was checked using a Beckman pH meter.

The reaction mixture usually consisted of 1.0 ml. sodium chloride solution with or without added inhibitor and buffered with .004 M sodium phosphate, 1.0 ml. stock soluble starch solution, and 0.5 ml. diluted enzyme solution. The exact constitution of the reaction mixture for each case is given in Tables I to IV. The time for digestion was usually 15 minutes. The mixtures were kept at constant temperature during the course of an experiment. Since controls were run for each experiment, and since only relative values for activity would suffice, modification of the digestion time could be made for separate experiments.

Enzyme activity is expressed in terms of milligrams of reducing sugar formed from 10 mg. of substrate. The method of Myers, Free, and Rosinski[15] was used in conjunction with a photoelectric colorimeter for determining reducing sugar.

The experiments concerned with the effects of high hydrostatic pressure involved the use of a specially designed pressure chamber. This consisted of a heavy walled, bored bronze cylinder tapped at one end for the attachment of brass tubing and threaded at the other to permit rapid attachment and removal of a heavy bronze cap. A Carver pressure pump, calibrated to register

*The term "denaturation" has been variously used. In this study, it is taken to denote a process characterized by a high heat of reaction and resulting in loss of specific biologic activity.

pressures up to 10,000 pounds per inch,[2] was attached to the distal end of the brass tubing. The entire system was oil filled. To apply pressure to a reaction mixture, the procedure consisted of adding the mixture to a 2 ml. vial, closing the vial with a rubber stopper without admitting air bubbles, dropping the stoppered vial into the oil-filled pressure chamber, attaching the cap, eliminating air from the oil line, placing the chamber in a temperature controlled water bath, and building up the pressure to the desired value. This entire procedure required 1¼ minutes. After the reaction mixture had been subjected to the pressure for the desired time, the pressure was released, the cap removed, and the contents of the vial were transferred to a test tube with 0.1 Gm. picric acid to stop enzymatic activity. This procedure also required 1¼ minutes.

RESULTS

Effects of Urea (5 to 9 Per Cent) and Ammonium Phosphate (0.1 Per Cent) at 37 to 39° C.—Urea (5 per cent or 0.83 M) is completely without inhibitory effect on salivary amylase activity in the presence of 10^{-2} N NaCl and 4 mg. per milliliter starch at 39° C. and pH 7. This is clearly shown by the data in Table I. For this experiment, no contact occurred between enzyme and urea prior to the start of digestion.

TABLE I. THE EFFECT OF TEMPERATURE ON THE ACTION OF UREA ON SALIVARY AMYLASE

REACTION MIXTURE						REDUCING SUGAR (MG./ML.)	% INHIBITION
INHIBITOR	STARCH	NaCl	AMYLASE	p_H	TREATMENT		
Moderate Temperature							
—	4.0 mg./ml.	1 × 10^{-2} N	+	7.0	39° C. Digestion 15 minutes	0.47	
Urea 5 % (0.83 M)			+			0.49	0
High Temperature							
—	4.0 mg./ml.	1 × 10^{-2} N	+	7.0	56° C. Digestion 16 minutes	0.63	
—			-			0.07	
Urea 5 % (0.83 M)			+			0.26	59
Urea 5 %			-			0.08	
—			+	6.7		0.78	
Urea 5 %			+			0.19	75

Prolonged contact with urea (8.7 per cent or 1.45 M) at 37 to 39° C. and pH 7 in the absence of starch does not affect amylase if .017 N NaCl is present. Thus the data in Tables II and III show no significant inhibition of activity during a 15-minute digestion period which followed a 20- to 60-minute period of contact between enzyme and 1.45 M urea in the presence of 0.017 N NaCl.

In the absence of sodium chloride and starch, amylase undergoes spontaneous, progressive inactivation at 37 to 39° C.[8, 10, 12] This is confirmed by the data in Table II. The presence of urea causes a marked increase in the rate of this inactivation. As shown in Table II, after 20-minute contact with 1.45 M urea, in the absence of salt and substrate, amylase inactivation at pH 7.2 may be increased as much as 70 per cent over that which occurs when urea, in addition to salt and substrate, is lacking. The degree of inactivation caused

by the action of urea under these circumstances is determined by comparing amylase activity in the presence and absence of urea in an ensuing period when NaCl and starch are present. That this effect of urea occurs only during the period when NaCl is lacking is indicated by the data, given in Table I, which establish that urea causes no inactivation at 37 to 39° in the presence of NaCl.

TABLE II. THE EFFECT OF UREA ON SALIVARY AMYLASE AT 39° C. IN THE ABSENCE OF STARCH AND WITH AND WITHOUT SODIUM CHLORIDE

REACTION MIXTURE					FINAL		REDUCING SUGAR (MG./ML.)	% INHIBITION
INHIBITOR	NaCl	AMYLASE	pH	TREATMENT	UREA	NaCl		
Standing at 39° C. With NaCl								
—		+		At 39° C. 20 min.	—	.007 N	0.47	
Urea 8.7 % (1.45 M)	.017 N	+	7.0	starch to give 3.0 mg./ml. then added. Digestion 15 min.	5%	.007	0.46	2
Urea 8.7 %	.017 N	-	7.0		5%	.007	0.04	
Standing at 39° C. Without NaCl								
—	.017 N	+		At 39° C. 20 min.	—	.007	0.45	
—	—	-		starch to give 3.0 mg./ml. and NaCl	—	.007	0.02	
—	—	+	7.2	to give	—	.007	0.30	
Urea 8.7 %	—	+		"Final NaCl" then added. Digestion 15 min. at 39° C.	5%	.007	0.09	70

The inactivation of amylase caused by the absence of NaCl and starch and the increase in the rate of inactivation by prolonged contact with urea under these conditions may be largely prevented by the presence of a low concentration (0.007 M or 0.1 per cent) of ammonium phosphate salts. (See Table III.)

TABLE III. EFFECT OF AMMONIUM PHOSPHATE ON INACTIVATION OF SALIVARY AMYLASE AT 37° C. IN THE ABSENCE OF SODIUM CHLORIDE AND STARCH

REACTION MIXTURE $_pH = 7.0$						REDUCING SUGAR (MG./ML.)	% INHIBITION
INHIBITOR	NaCl	$NH_4H_2PO_4$ $(NH_4)_2HPO_4$	AMYLASE	TREATMENT	FINAL NaCl		
—	.017 N	—	+	At 37° C. 60 min.	.007	0.40	
—	—	—	+	starch to give	.007	0.10	
Urea 8.7 % (1.45 M)	.017 N	—	+	3.0 mg./ml. then added.	.007	0.40	0
Urea 8.7 %	—	.007 M	+	H_2O or NaCl	.007	0.38	5
—	—	.007 M	+	Added to give "Final (NaCl)"	.007	0.35	12
—	—	.007 M	+	Digestion 11 min. 37° C.	0.0	0.03	93

The Effects of Urea at 50 to 60° C.—The effect of urea on amylase being spontaneously inactivated by standing at 37 to 39° in the absence of salt and substrate suggests that urea would act on amylase undergoing inactivation or denaturation in other conditions.

One phase of amylase heat inactivation is rapidly reversible by a reduction in temperature.[10] This process of reversible inactivation or denatura-

tion is appreciable in rate even at 37° C. and in the presence of 10^{-2} N NaCl.[12] The absence of any apparent effect of urea at this temperature in the presence of NaCl, however, makes it doubtful that urea exerts any appreciable effect on this reversible denaturation process (Table I).

The enzyme undergoes a spontaneous inactivation or denaturation at temperatures above the optimum, even in the presence of NaCl, which is irreversible in the sense that activity is not readily restored by lowering the temperature.[12] This process is not appreciable at 37° in the presence of NaCl and substrate. The effects of urea on this inactivation were investigated at temperatures above 50° in the range 50 to 60° C. (The temperature optimum for salivary amylase activity varies between 40 and 50° C.; its exact position depends on the concentration of starch, NaCl, and other antidenaturant substances in the reaction mixture.[8, 10, 16])

At 50° C., 5 per cent urea was found to inhibit amylase activity 11 per cent (Table IV) at pH 7. At 56°, a point at which considerable temperature-irreversible denaturation is known to occur,[12] 5 per cent urea caused 59 to 75 per cent inhibition at pH 6.7 and 7.0. This is shown in Table I.

The effects of high hydrostatic pressure at 50° further indicate that the high temperature action of urea is not mediated by an effect on the reversible denaturation process but is associated with the apparently irreversible denaturation.

High hydrostatic pressure counteracts the volume increase of denaturation and thereby reduces the rate of the process. This effect is probably mediated largely by the reversible phase of the denaturation,[13] and at temperatures where the rate of the reversible process is appreciable, hydrostatic pressure thereby increases the apparent activity of the enzyme.[14] Thus, as shown in Table IV, at 50° C., the application of 5500 pounds per inch^2 pressure increased amylase activity 30 per cent in the absence of urea. However, as shown in Table IV, with 5 per cent urea present, application of the same degree of pressure increased amylase activity only 12 per cent, and consequently no reversal of the inhibition by pressure is apparent.

TABLE IV. THE EFFECT OF HIGH HYDROSTATIC PRESSURE ON THE INHIBITION OF AMYLASE BY UREA AT HIGH TEMPERATURE WITH STARCH AND NaCl PRESENT

REACTION MIXTURE				P = 15 LB./IN.^{2}		P = 5500 LB./IN.^{2}		%
INHIBITOR	STARCH	NaCl	°C.	REDUCING SUGAR MG./ML.	% INHIBITION	REDUCING SUGAR MG./ML.	% INHIBITION	CHANGE BY PRESSURE
—	4.0	.01 N		0.73		0.95		
Urea 5.%	mg./ml.	.01 N	50	0.65	11	0.73	23	+12

At 50° C., both reversible and irreversible inactivation of amylase occurs. If the inhibition by urea were mediated by an action on the reversible denaturation process, it is to be expected that pressure would reduce this inhibition.[17, 18, 19] The inhibition by urea at high temperatures, therefore, appears to be mediated by the process of denaturation which is not readily

reversible. This is further substantiated by the concomitant increase in inhibition by urea and proportion of irreversibly inactivated amylase which occurs with an increase in temperature above 50° C.

DISCUSSION

Urea, in concentrations lower than 2 M, has been reported to be without effect on β amylase.[20, 21] The data reported here indicate clearly that urea in concentrations up to 1.45 M (8.7 per cent) is without inhibitory effect on salivary α amylase under conditions which do not lead to inactivation of the enzyme. Urea appears to have no effect on reversibly denatured salivary amylase as judged by the absence of inhibition at 39° C. in the presence of NaCl and the inability of pressure to reverse the inhibition by urea at 50° C.

Under conditions which lead to progressive, not readily reversible inactivation or denaturation, urea causes marked inhibition of amylase. This occurs during prolonged standing at 37 to 39° in the absence of antidenaturant salts and during short periods of contact between urea and amylase at temperatures above the optimum for amylase activity, even in the presence of antidenaturant salts. These effects are similar to those caused by the inhibitors iodoacetic acid, quinine, and urethane on salivary amylase,[14, 22] and also resemble, in part, an effect of urethane on spore disinfection.[23]

Sodium chloride affects amylase in two ways: first, a halide salt is required for activity of amylase, and NaCl is an effective activator for amylase.[8] In this action, the effect is mediated by the halide ion.[16] Second, the presence of the halide salt protects the amylase against spontaneous inactivation or denaturation, and this action is mediated largely by the cation.[8, 12] The data presented here indicate that, while the phosphate ion cannot activate amylase, the ammonium ion does protect against denaturation almost to the same extent as the Na^+ ion. The ammonium ion is capable also of preventing the low temperature inhibition of urea in the absence of substrate and probably does this by preventing the spontaneous inactivation of the enzyme. From the data presented here, it appears likely that this inactivation must precede any action of urea.

Limitation of the action of urea to an effect on the progressive, spontaneous inactivation processes occurring at high temperature or on prolonged standing at low temperature in the absence of protective salts indicates that urea-reactive groups of the enzyme become available in these inactivation processes. The release of reactive groups, probably NH_2, in the inactivation process has been indicated by other work on amylase.[14]

It has been indicated that the use of a solution containing 13 per cent urea and 3 per cent diammonium phosphate as an oral rinse raises the pH of the tooth surface to as much as 8.0.[24] Although most of the solutions employed in the present investigation were adjusted to pH 7.0, some observations were made at pH 8. Within the pH range 6.7 to 8.0, no appreciable effect of pH changes on the action of urea was observed. This is to be expected, since at pH 8, salivary amylase is 90 per cent as active as at the optimal pH of 6.9.[16, 25] In addition, salivary amylase is stable within the pH range 4.8 to 11.[16, 25]

The results of this investigation of the action of urea indicate that this chemical agent would be without effect on amylase in vivo in concentrations resulting from the use of ammoniated dentifrices, even under conditions of frequent or prolonged use. With amylase, at least, the inactivating action of 5 to 9 per cent urea becomes significant only under conditions which themselves lead to enzyme inactivation of a progressive type. This inactivation does not occur to any appreciable extent at 37 to 39° C. at pH 6.7 to 8.0 and in the presence of .007 M ammonium phosphates or with the concentration of sodium and potassium salts present in the saliva normally. Whether urea, in the concentrations resulting from the use of available dentifrices, affects the caries process through a denaturing action, still cannot, of course, be stated, since enzymes other than amylase may well be involved. On the basis of this study of the mode of action of urea on amylase, such a mechanism might, however, appear doubtful.

SUMMARY

Urea in a concentration of from 5 to 9 per cent (0.83 to 1.45 M) is without inhibitory effect on salivary amylase at 37 to 39° C. and pH 7 in the presence of 10^{-2} N sodium chloride.

At high temperatures (above 50° C.) and at low temperatures in the absence of NaCl, amylase undergoes spontaneous, progressive inactivation or denaturation. Under these conditions, 5 to 9 per cent urea markedly inhibits amylase, apparently by an effect on the denaturation process.

The inhibitory effect of urea is counteracted by 0.1 per cent (.007 M) ammonium phosphate.

It is unlikely that urea in concentrations resulting from the use of commercially available dentifrices affects the caries process in vivo by means of a denaturing action on amylase.

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THE EFFECTIVENESS OF AN AMMONIUM ION TOOTH POWDER IN THE CONTROL OF DENTAL CARIES

II. Composition of Dentifrices Used in the Experiment

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IN A previous paper[1] we published the results of an experiment which was designed to determine the effectiveness of an ammonium ion tooth powder when used without supervision during the period March to November, 1950. In discussing the results obtained we stated that our experimental powder was the same as Dentifrice B used by Kerr and Kesel.[2] This is not correct. Our experimental and control powders contained 5 per cent bentonite whereas the dentifrices used by Kerr and Kesel did not contain bentonite.

To obviate any misunderstanding in the interpretation of our results, the formulas of the respective experimental and control powders are reported here.

Table I
Experimental Tooth Powders

	Quantities to make 10 kg.	
	Experimental powder of Davies and King (gm. or c.c.)	Experimental powder of Kerr and Kesel (dentifrice B) (gm. or c.c.)
Dibasic ammonium phosphate	500	500
Urea	300	300
Bentonite	500	—
Soluble saccharin	30	20
Menthol	20	20
Calcium carbonate precip.	8350	9160
Sodium lauryl sulfate	200	100
Oil of peppermint	20	20
Oil of wintergreen	—	60
Oil of cinnamon	—	19
Methyl salicylate	80	—

Table II
Control Tooth Powders

	Quantities to make 10 kg.	
	Control powder of Davies and King (gm. or c.c.)	Control powder of Kerr and Kesel (dentifrice A) (gm. or c.c.)
Soluble saccharin	30	20
Oil of peppermint	20	20
Menthol	20	20
Oil of wintergreen	—	60
Oil of cinnamon	—	19
Methyl salicylate	80	—
Sodium lauryl sulfate	200	100
Calcium carbonate	9150	9960
Bentonite	500	—
Carmine (to color)	16	

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THE ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX

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WITHIN recent times, at least 3 groups of investigators[1-5] have supported the concept that dental caries is primarily the result of the action of lytic bacteria upon the organic matrices of enamel and dentin. Experimental evidence to support this theory is sparse and inconclusive in comparison to the amount of accumulated data which indicate that acidogenic bacteria or their products function in the dental carious process. The latter studies are too numerous and well known to be reviewed in this introduction. With regard to the role of proteolytic bacteria in the carious process, the accumulated experimental evidence indicates that the organic matrix of intact enamel or dentin is not readily accessible for destruction by proteolytic enzymes of bacterial origin. Miller[6] isolated proteolytic microorganisms from saliva and from the carious lesion but these bacteria produced no gross change in intact enamel or dentin exposed to them in vitro. Rodella[7] claimed that the entire decomposition process in dental caries was due to a putrefactive, anaerobic bacterium, *Bacillus putrificus,* that also formed acid from several carbohydrates. The proteolytic bacteria isolated from the carious lesion by Gies and his associates[8-12] could not lyse intact dentin in vitro. Earlier, Goadby[13] had isolated several types of bacteria from the carious lesion which could liquefy decalcified dentin exposed to them in broth or on agar slant cultures. However, these bacteria had no apparent effect on intact dentin or enamel. McIntosh, James, and Lazarus-Barlow[14, 15] found strains of *Staphylococcus albus* and *Staphylococcus aureus* that could lyse decalcified, but not intact, dentin. They also found that Types I and II of *Bacillus acidophilus-odontolyticus* could produce liquefaction foci in the pulpal side of dentin exposed to them in vitro. The findings of McIntosh, James, and Lazarus-Barlow led Hadley[16] to postulate that the oral lactobacilli produced a proteolytic enzyme that could liquefy the organic matrix of dentin but this contention has not been validated. Bibby[17] found that ordinary proteolytic bacteria could not disintegrate sections of intact enamel. Pincus[18] produced enamel lesions, presumably by proteolytic action, by the use of unidentified oral bacteria, in vitro, at pH 7.2 to 7.4. In other studies, however, Pincus[2] found that keratinolytic bacteria, effective against many types of keratin, were unable to affect the organic matrix of intact enamel. The findings of the previous investigations,

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therefore, indicate that proteolytic bacteria are present in both the oral cavity and in the carious lesion that can lyse decalcified dentin but which, acting alone, cannot affect intact dentin. Apparently certain changes occur in the organic matrix of dentin during decalcification or in the early stages of dentinal caries which make it accessible to the action of proteolytic enzymes. The nature of these changes is not completely understood. The present investigation, therefore, is concerned with the development of a method for measuring the accessibility of dentinal protein for proteolytic enzymes under conditions such as might be found in the carious process, and with the application of such a method to the study of intact and carious dentin, and acid-treated and heat-treated dentin. The results of the application of this method to dentin exposed to oral bacteria and to proteolytic enzymes will be presented in a subsequent paper.

METHODS

Bergmann and Fruton,[19] in their studies of the specificity of the proteolytic enzymes, found that accessible tyrosine must be present in the protein chain adjacent to the dipeptide linkage before pepsin or carboxypeptidase could break such linkages. When proteins containing accessible tyrosine and histidine residues are exposed to diazohydroxides, coupling occurs in the manner depicted in Fig. 1, with the formation of colored azoproteins. The chemistry of this reaction is outlined in Fig. 2, showing the course of this reaction with tyrosine. With proper variations, this reaction has been widely utilized in immunology for the preparation of artificial antigens. Charney and Tomarelli[20] also employed this reaction to prepare a protein substrate for use in the measurement of the rate of proteolysis by various enzymes from the intestinal tract. We have found that this reaction may be applied as an indicator of the accessibility of the organic (i.e., protein) matrix of dentin during caries or after exposure to acids or bacteria.

In the present study, a modification of the method of Charney and Tomarelli[20] for preparing azoprotein was evolved. The diazohydroxide employed to react with the organic components of dentin was prepared as follows: 5 Gm. of sulfanilamide were dissolved in 200 ml. of distilled water containing 6 ml. of 5N NaOH, and the solution was cooled to from 0° to 5° C. Sodium nitrite (2.2 Gm.) was added and dissolved with stirring. Eighteen ml. of 5N HCl, cooled to 0° C., were then added and the solution was stirred for at least 2 minutes. Eighteen ml. of 5N NaOH, cooled to 0° C., were then added and the solution was stirred for at least 2 minutes. The final solution containing the diazohydrate was a light wine color with a pH of from 8.0 to 8.5. Five ml. amounts of the diazohydroxide solution were then added to test sections of dentin in individual test tubes, chilled to from 0° to 5° C. After 24 hours at 0° C., the supernatant solution was decanted and the diazotized dentin sections were washed in distilled water until no more color could be removed. As a measure of the degree of coupling, the azoprotein that had formed was removed by treating the sections with 1N NaOH at 65° C. for 30

minutes. The red or reddish-orange color released was measured in a Klett-Summerson colorimeter using filter No. 42. As controls, intact dentin sections not diazotized were extracted in a similar manner.

After a satisfactory standard procedure had been evolved, the amounts of accessible protein were determined in various types of dentin samples, namely: in intact, untreated dentin; in acid decalcified and naturally carious dentin; in dentin exposed to moist heat; and in dentin partially decalcified by organic and inorganic acids (a) in Douglas' broth brought to pH 3.0 by addition of the respective acids without regard to their concentration and (b) in Douglas' broth containing the respective acids in a fixed molar concentration, but adjusted to pH 3.0 by the addition of sodium hydroxide or hydrochloric acid as required.

RESULTS

Diazotization was first attempted with sections of intact and acid-decalcified dentin. Freshly extracted teeth were cut just below the dentino-enamel junction by the use of carborundum disks. Some of the sections were diazotized intact, some after partial decalcification, and others after complete decalcification in Morse's solution.[21] Typical examples of the results obtained are shown in Fig. 1. The organic matrix of the intact, untreated dentin did not react visibly with diazohydroxide, whereas the protein of the pulp reacted to form reddish azoprotein. As dentin was progressively decalcified, it was apparent that it reacted more readily with the diazohydroxide to form increasing amounts of azoprotein, indicating that tyrosine in the matrix became accessible as a result of the action of the decalcifying acid. Sections of dentin, dried to a constant weight over calcium chloride at 37° C., reacted in a similar manner to sections of dentin from freshly extracted teeth. Consequently, in the following experiments, dentin sections, with all extraneous organic material removed and dried to a constant weight over calcium chloride at 37° C., were utilized.

If the formation of a colored azoprotein was to be used as a valid measure of accessible dentinal protein, it was essential to establish that a constant amount of alkali-soluble color (azoprotein) was formed per unit weight of the dentinal protein remaining after decalcification. Accordingly, 35 sections of dentin (7 from each of 5 teeth) were decalcified as completely as possible in a buffered formic acid solution at pH 3.0 (Morse's decalcifying solution[21]) for 28 days, with changes of solution each week. Three sections were lost accidentally during decalcification. The residual material, essentially dentinal protein, was air-dried to a constant weight and then treated with diazohydroxide as described. The results are summarized in Table I and are also depicted graphically in Fig. 3 for comparison with naturally carious dentin treated under similar circumstances. Not only did the amounts of color (azoprotein) per milligram of residual matrix of the sections of each tooth fall within a narrow range, but the combined values for all of the sections of different teeth had a coefficient of variation of only 10.7 per cent, which is scarcely

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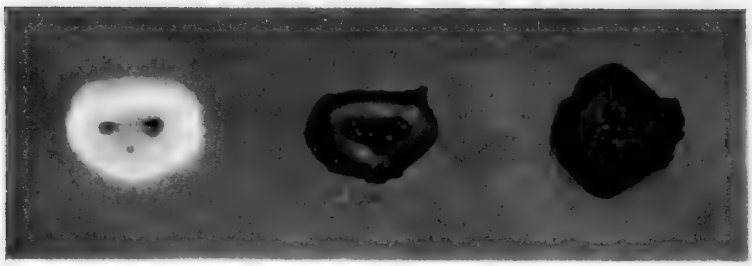
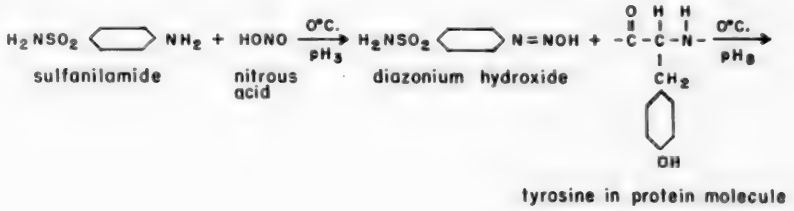


Fig. 1.—Photograph of different types of diazotized dentin. The section on the left is intact dentin; the center section was decalcified 24 hours in Morse's decalcifying solution before exposure to diazohydroxide; the section on the right was completely decalcified in Morse's decalcifying solution prior to diazotization.

greater than the possible error in weighing the small samples that were utilized. The standard error of the mean of all values was 0.94 and the ratio of the mean to its standard error was 53.0. Therefore, it was concluded that the amount of color formed was a valid measure of the accessible dentinal protein.



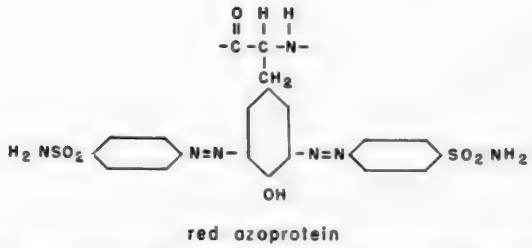


Fig. 2.—Diazotization of protein containing accessible tyrosine.

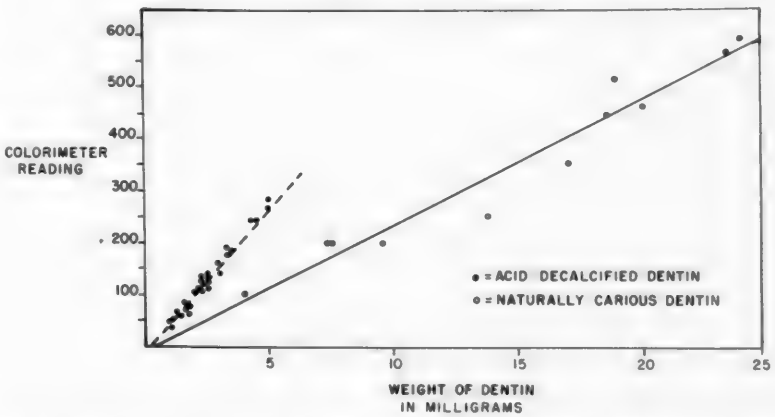


Fig. 3.—Comparison of rate of diazotization of residual material of completely acid-decalcified material and naturally carious dentin.

The next objective was to determine the rate of diazotization of naturally carious dentin. Eleven samples of dentin, removed from carious lesions that had reached the decalcification stage, were dried, weighed, and diazotized. The results of this test are summarized in Table II and are shown graphically in Fig. 3 for comparison with acid-decalcified dentin, diazotized under

TABLE I

THE DIAZOTIZATION OF COMPLETELY ACID-DECALCIFIED DENTIN*

TOOTH NO.	NO. OF SAMPLES	MEAN DRY WEIGHT (MG.)	MEAN COLORIMETER READING	MEAN COLORIMETER† READING/MG.
1	7	3.3	170	50.1
2	6	1.8	98	51.3
3	7	2.3	114	48.1
4	6	2.2	107	46.4
5	6	2.6	144	53.5

*Decalcified 28 days in Morse's decalcifying solution.

†Mean of all values, 50.0; standard deviation, 5.36; coefficient of variation, 10.7 per cent; standard error of mean, 0.94; mean ÷ standard error of mean, 53.0.

similar circumstances. The mean colorimeter reading per milligram of weight of the samples of naturally carious dentin was 23.3, with a standard deviation of 2.78 and a coefficient of variation of 12.0 per cent. The ratio of the mean to its standard error was 27.7. The amount of azoprotein formed by the naturally carious dentin, therefore, was also a reproducible measure of the accessible dentinal protein. A comparison of the rate of diazotization of acid-decalcified dentin with that of carious dentin at the decalcification stage shows, however, that twice as much color per unit weight is produced by the former as by the latter.

TABLE II

THE DIAZOTIZATION OF NATURALLY CARIOUS DENTIN

SECTION NUMBER	DRY WEIGHT	COLORIMETER READING	COLORIMETER READING/*MG. DRY WEIGHT
1	0.0188	440	23.4
2	0.0236	560	23.7
3	0.0139	250	17.9
4	0.0202	460	22.7
5	0.0075	196	26.1
6	0.0076	196	25.8
7	0.0242	588	24.2
8	0.0191	520	27.2
9	0.0040	100	25.0
10	0.0096	200	20.8
11	0.0172	350	20.3

*Mean of all values, 23.3; standard deviation, 2.78; coefficient of variation, 12.0 per cent; standard error of mean, 0.84; mean ÷ standard error of the mean, 27.7.

Intact, untreated sections of dentin were tested next since it had been found previously that their organic matrices did not react with diazohydroxide and were not accessible to the lytic enzymes of oral bacteria. Ten thin sections of dentin, with a mean weight of 0.0235 Gm., were diazotized and the amount of azoprotein produced was measured. The results are summarized in Table III for comparison with heat-treated dentin. During the process of diazotization, the mean weight loss of these sections of dentin was 6.47 per cent. The diazohydroxide reacted with the intact sections of dentin to give a mean colorimeter reading per milligram of total weight of only 0.62, with a standard deviation of 0.18. An examination of the dentin sections, after diazotization and before removing the color, indicated that the diazohydroxide appeared to couple to a small amount of organic material lining the root canal and to a slight extent to material in the dentinal tubules.

Since the organic matrix of decalcified dentin can be broken down or converted to gelatin by boiling, 7 sections of intact dentin, similar to those used in the previous experiment, were placed in boiling water for 30 minutes, and an equal number were autoclaved at 120° C. for 20 minutes, to determine if such treatment might make the organic matrix accessible for chemical reactivity. After exposure to moist heat, the sections were diazotized. The results of these experiments are shown in Table III. The mean weight of the dentin sections was 0.0285 Gm. before exposure to moist heat at 100° C. After exposure and diazotization, they gave a mean colorimeter reading per milligram of total weight of only 1.16, with a standard deviation of 0.94. The percentage weight loss was only 2.44 in contrast to 6.47 for untreated dentin sections. The average weight of the dentin sections which were to be autoclaved was 0.0166 Gm. After autoclaving and exposure to the diazohydroxide, they gave a mean colorimeter reading per milligram of total weight of 1.44, with a standard deviation of 0.85. The mean percentage weight loss of these sections was 9.87.

TABLE III

THE DIAZOTIZATION OF VARIOUS TYPES OF INTACT DENTIN

TYPE OF DENTIN	NO. OF SAMPLES	MEAN WT. OF SAMPLES	MEAN COLORIMETER READING	MEAN COLORIMETER RDG./MG.	SD	PER CENT LOSS IN WT.
Intact	10	0.0235	14	0.62	0.18	6.47
Boiled	7	0.0285	28	1.16	0.94	2.44
Autoclaved	7	0.0166	22	1.44	0.85	9.87

Since variable weight losses were experienced by dentin during the various types of treatments, an attempt was made to determine whether they were caused by the procedures carried out during diazotization. Four sections of intact, untreated dentin were extracted with the 1N NaOH for 30 minutes at from 60° to 65° C. The extracted sections had a mean weight loss of 1.8 per cent in contrast to the loss of from 3 to 10 per cent during the entire process of diazotization. Thus, it appears that the diazotization procedures per se contribute, in part, to the total weight lost during the test.

TABLE IV

THE RELATIONSHIPS BETWEEN DECALCIFICATION WITH NONEQUIMOLAR ACIDS AND THE ACCESSIBILITY OF THE ORGANIC MATRIX OF DENTIN

TOOTH NO.	NO. OF SECTIONS	MEAN WT. (GM.)	MEAN COLORIMETER READING	COLORIMETER READING/MG. TOTAL WT.	MEAN % LOSS IN WT.	COLORIMETER* READING/MG. WT. LOSS
1	7†	0.0301	146	4.9	29.5	17.8
2	6†	0.0323	129	4.1	25.9	15.9
3	7†	0.0354	174	4.8	26.5	18.5
4	7†	0.0345	170	5.7	33.7	17.9
5	7†	0.0330	184	6.0	31.2	21.5

*Mean of all values, 18.42; standard deviation, 3.16; coefficient of variation, 17.2 per cent; mean ÷ standard error of the mean, 34.1; coefficient of correlation, 0.95.

†The sections of each tooth were decalcified for 24 hours in Douglas' broth adjusted with lactic, acetic, butyric, formic, nitric or hydrochloric acid to pH 3.0 or with succinic acid to pH 4.0, respectively.

The next objective was to determine if exposure to various organic and inorganic acids released the organic matrix of dentin for diazotization in proportion to the degree of decalcification. Seven sections of dentin were cut from each of 5 teeth. Only 6 sections of dentin of tooth 2 were considered in the final calculations. Each group, representing 1 tooth, was decalcified for 24 hours at 37.5° C. in Douglas' broth adjusted with lactic, acetic, butyric, formic, nitric, or hydrochloric acid to pH 3.0 and with succinic acid to pH 4.0, after which the sections were diazotized and the color formed was measured.

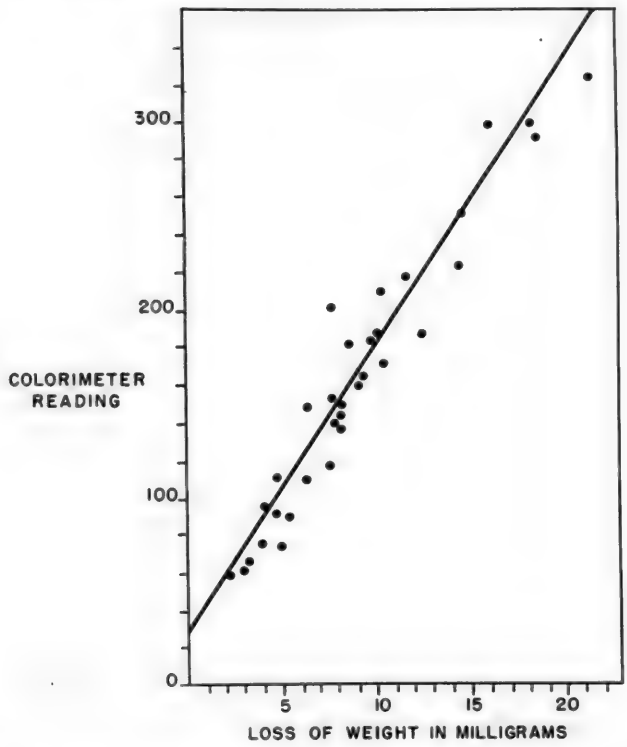


Fig. 4.—Rate of diazotization of 34 sections of dentin from 5 teeth following decalcification in nonequimolar acids for 24 hours at 37° C. Regression equation is $Y = 26.6 + 14.76X$. Coefficient of correlation between degree of diazotization and weight loss is 0.95.

The results are shown in Table IV and Fig. 4. The colorimeter reading obtained for each section varied according to the weight of the sample and to the type of acid used for decalcification. The mean percentage loss in weight for the sections from each of the 5 teeth was 29.5, 25.9, 26.5, 33.7, and 31.2, with a corresponding mean colorimeter reading per milligram of weight loss of 17.8, 15.9, 18.5, 17.9, and 21.5. The mean of all the colorimeter readings was 18.42 with a standard deviation of 3.16 and a coefficient of variation of 17.2 per cent. The coefficient of correlation between the loss in weight and the accessibility of the organic matrix was 0.95. The relationship between loss in weight and

colorimeter reading is shown graphically in Fig. 4. The regression equation for these values was $Y = 26.6 + 14.76\ X$, giving the regression line shown. This line does not pass through the zero point of the Y axis for, as has already been shown, a small amount of diazotization occurs in intact dentin.

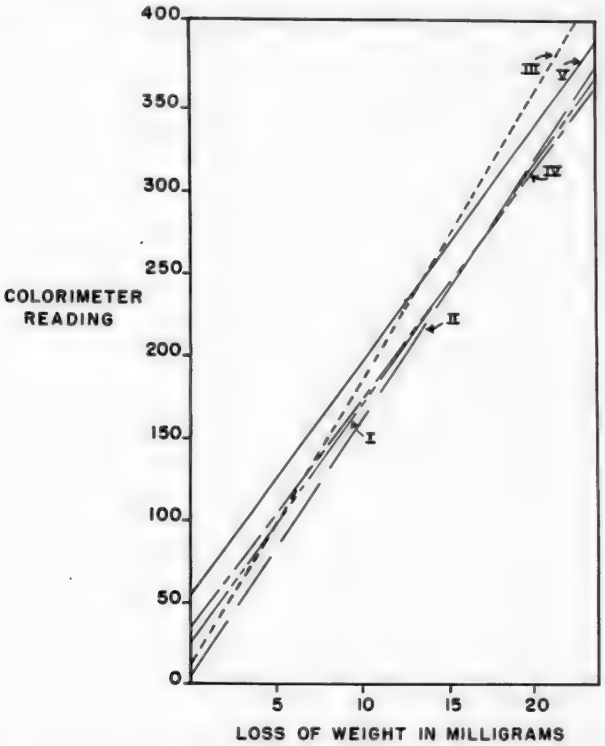


Fig. 5.—Rate of diazotization of dentin sections from 5 teeth after decalcification in non-equimolar acids for 24 hours at 37° C. Regression equations for individual teeth are: (1) $Y = 24.88 + 14.04X$; (2) $Y = 5.22 + 15.27X$, (3) $Y = 12.71 + 16.89X$, (4) $Y = 35.41 + 13.57X$, (5) $Y = 52.17 + 14.07X$.

Regression equations were then calculated for each tooth and each regression line was plotted in Fig. 5. Since these regression lines varied slightly between individual teeth, the data for each tooth were analyzed statistically to see if a real difference existed between individual teeth in the amount of protein made accessible per unit of decalcification. However, it was necessary to make certain corrections in the data before analyzing them. Since the amount of inherently accessible protein varied from tooth to tooth, it was necessary to recalculate the values of the colorimeter reading per milligram of weight loss for each tooth by subtracting the value of the intercept of the particular regression line on the Y axis. The corrected values then represented the actual amount of protein made accessible by decalcification. The statistical analysis of the corrected data is shown in Table V. The critical ratios (difference between the means divided by its standard error) for the

differences in the amount of accessible protein per unit decalcification of the individual teeth are not greater than two for any teeth except I versus III, II versus III, II versus IV, and III versus IV.

TABLE V

AN ANALYSIS OF THE CORRECTED DATA OF THE RATE OF DIAZOTIZATION FOLLOWING DECALCIFICATION BY NONEQUIMOLAR ACIDS

TOOTH NO.	NO. OF SECTIONS	CORRECTED MEAN COLORIMETER RDG./ MG. WT. LOST	SD	CV	SEM	M/SEM
1	7	13.91	2.90	20.8	1.09	12.76
2	6	15.23	1.21	7.9	0.49	31.08
3	7	16.80	1.37	8.1	0.51	32.94
4	7	13.53	1.08	7.9	0.40	33.82
5	7	13.08	4.91	37.5	1.85	7.07

Critical ratios between various teeth.

I versus II = 1.1	II versus III = 2.2	III versus IV = 5.0
I versus III = 2.4	II versus IV = 2.6	III versus V = 1.93
I versus IV = 0.32	II versus V = 1.1	IV versus V = 0.23
I versus V = 0.38		

The sections of dentin in the above experiment were decalcified in solutions of Douglas' broth adjusted to a given pH, but the molarity of the weak and the strong acids varied considerably. Under these conditions the various acids decalcified at different rates (Table VI). If succinic acid is not considered (pH 4.0), the critical ratios for the differences in the degrees of decalcification by the other acids are greater than two in all cases except lactic versus acetic, acetic versus formic, butyric versus hydrochloric, and succinic versus nitric. It is apparent that some acids may decalcify at different rates even though at the same pH.

TABLE VI

A SUMMARY OF THE RATE OF DECALCIFICATION BY NONEQUIMOLAR ACIDS*

TYPE OF ACID	MEAN % DECALCIFICATION	SD	CV	SEM	M/SEM
Lactic	35.5	10.43	29.3	4.65	7.71
Acetic	42.5	8.67	20.4	3.87	11.18
Butyric	24.7	3.72	15.0	1.66	14.87
Succinic	14.7	3.89	26.4	1.73	8.64
Formic	55.4	15.74	28.4	7.02	7.91
Nitric	10.6	3.62	34.1	1.61	6.62
Hydrochloric	23.7	5.55	23.4	2.47	9.87

*All acids pH 3.0 except succinic (pH 4.0); decalcification time, 24 hours.

Critical ratios between the decalcification rates of different acids.

Lactic versus acetic	1.0	Butyric versus succinic	4.2
Lactic versus butyric	2.2	Butyric versus formic	4.2
Lactic versus succinic	4.2	Butyric versus nitric	6.2
Lactic versus formic	2.3	Butyric versus hydrochloric	0.3
Lactic versus nitric	5.1		
Lactic versus hydrochloric	2.2	Succinic versus formic	5.6
		Succinic versus nitric	1.7
Acetic versus butyric	4.3	Succinic versus hydrochloric	3.0
Acetic versus succinic	6.6		
Acetic versus formic	1.6	Formic versus nitric	6.2
Acetic versus nitric	7.7	Formic versus hydrochloric	4.2
Acetic versus hydrochloric	4.1		
		Nitric versus hydrochloric	4.5

In order to determine whether the difference in the rate of decalcification of the various acids was due to qualitative effects of the respective anions, an experiment was set up in which the acids were added to Douglas' broth to

make a 0.1 molar solution. A 0.1 molar lactic acid-Douglas' broth mixture gave a pH of approximately 3.0 and was used as a basic reference point. The 0.1 molar solutions of the other acids varied slightly from this figure. All solutions were adjusted to pH 3.0 by the addition of a small amount of either concentrated HCl or NaOH as necessary.

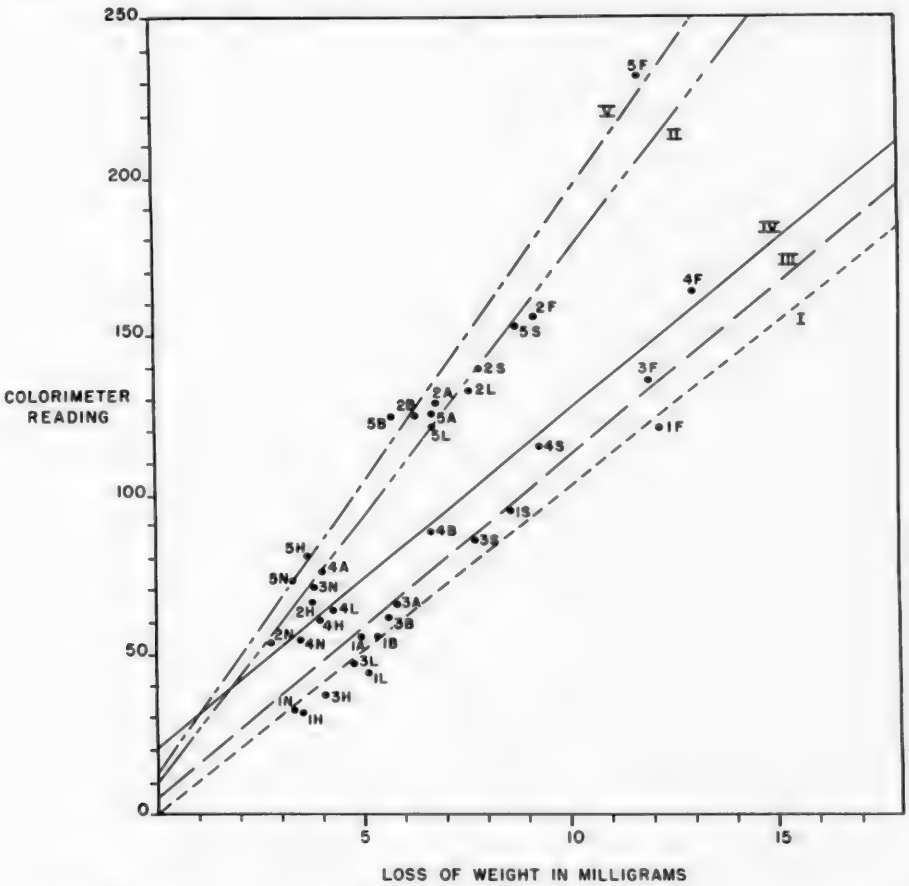


Fig. 6.—Rate of diazotization of dentin sections from 5 teeth after decalcification in equimolar acids for 24 hours at 37° C. Individual sections of each tooth are numbered from 1 to 5. Decalcifying acids are designated: *L*, lactic, *A*, acetic, *B*, butyric, *S*, succinic, *F*, formic, *N*, nitric, *H*, hydrochloric. The regression equations for the individual teeth are: (1) $Y = 0.78 + 10.25\times$, (2) $Y = 10.49 + 16.60\times$, (3) $Y = 4.80 + 10.76\times$, (4) $Y = 20.25 + 10.72\times$, (5) $Y = 12.00 + 18.14\times$.

Sections of dentin were prepared as described in the previous experiment and decalcified with the equimolar acid-Douglas' broth mixtures. The results of this experiment are summarized in Table VII and Fig. 6. The coefficient of correlation between weight loss and the amount of diazotization was 0.78. While still significant, this value was less than that in the previous experiment using nonequimolar acids at a constant pH only. There was considerable

scattering of the values when they were plotted graphically in Fig. 6. For this reason, regression equations were calculated for each tooth giving the regression lines shown.

TABLE VII

THE RELATIONSHIP BETWEEN DECALCIFICATION BY EQUIMOLAR ACIDS AND THE ACCESSIBILITY OF THE ORGANIC MATRIX OF DENTIN

TOOTH NO.	NO. OF SECTIONS	MEAN TOTAL WT. (GM.)	MEAN COLORIMETER RDG.	COLORIMETER READING/MG. TOTAL WT.	MEAN % LOSS IN WEIGHT	COLORIMETER RDG./MG. WT. LOSS*
1	7	0.0299	62	2.14	20.77	10.30
2	7	0.0335	114	3.62	20.32	18.04
3	7	0.0329	722	2.47	22.25	11.74
4	7	0.0370	89	2.66	18.72	14.70
5	7	0.0375	133	3.59	17.62	20.22

*Mean of all values, 15.00; standard deviation, 4.18; coefficient of variation, 27.9 per cent; mean ÷ standard error of the mean, 21.0; coefficient of correlation (r), 0.78.

Since these regression lines apparently varied between individual teeth, the data for each tooth were analyzed statistically to see if a real difference existed between individual teeth in the amount of protein made accessible per unit of decalcification. The data were first corrected in the same fashion used in the analysis of the data presented in Table IV and Fig. 5 by subtracting the value of the intercept of the particular regression line on the Y axis. The statistical analysis of the corrected data is shown in Table VIII. The critical ratios for the differences in the amount of accessible protein per unit decalcification of the individual teeth are greater than two for all comparisons except those between teeth I, III, and IV.

TABLE VIII

AN ANALYSIS OF THE CORRECTED DATA OF THE RATE OF DIAZOTIZATION FOLLOWING DECALCIFICATION BY EQUIMOLAR ACIDS

TOOTH NO.	NO. OF SECTIONS	CORRECTED MEAN COLORIMETER RDG./ MG. WT. LOSS	SD	CV	SEM
1	7	10.18	1.01	9.9	0.38
2	7	16.14	1.05	6.5	0.40
3	7	10.88	3.19	29.3	1.20
4	7	10.76	1.44	13.4	0.54
5	7	18.15	1.46	8.04	0.55

Critical ratios between various teeth.

I versus II = 10.8		II versus IV = 8.0	
I versus III = 0.55		II versus V = 2.9	
I versus IV = 0.87		III versus IV = 0.09	
I versus V = 11.9		III versus V = 5.5	
II versus III = 4.1		IV versus V = 9.6	

In addition, a statistical analysis of the decalcification rates of the various equimolar acids have been summarized in Table IX. The critical ratios for the differences in the degree of decalcification by the various acids are greater than two in all cases except lactic versus succinic, acetic versus butyric and nitric versus hydrohloric.

TABLE IX

A SUMMARY OF THE STATISTICAL ANALYSIS OF THE RATE OF DECALCIFICATION BY EQUIMOLAR ACIDS*

TYPE OF ACID	MEAN % DECALCIFICATION	SD	CV	SEM	M/SEM
Lactic	26.4	5.04	19.1	2.25	11.73
Acetic	18.2	4.45	24.4	1.98	9.19
Butyric	17.3	2.80	16.1	1.25	13.88
Succinic	26.9	1.31	4.8	0.58	46.37
Formic	33.2	3.82	11.4	1.70	19.56
Nitric	8.9	2.06	23.1	0.91	9.78
Hydrochloric	8.4	0.73	8.7	0.32	26.25

*All acids pH 3.0; decalcification time, 24 hours.

Critical ratios between the various acids.

Lactic versus	acetic	=	2.7	Butyric versus	succinic	=	6.9
Lactic versus	butyric	=	3.5	Butyric versus	formic	=	7.5
Lactic versus	succinic	=	0.21	Butyric versus	nitric	=	5.4
Lactic versus	formic	=	2.4	Butyric versus	hydrochloric	=	6.9
Lactic versus	nitric	=	7.2				
Lactic versus	hydrochloric	=	7.9	Succinic versus	formic	=	3.5
				Succinic versus	nitric	=	16.7
Acetic versus	butyric	=	0.38	Succinic versus	hydrochloric	=	27.8
Acetic versus	succinic	=	4.2				
Acetic versus	formic	=	5.7	Formic versus	nitric	=	12.2
Acetic versus	nitric	=	4.2	Formic versus	hydrochloric	=	14.4
Acetic versus	hydrochloric	=	4.8				
				Nitric versus	hydrochloric	=	0.51

DISCUSSION

The question of whether or not the organic matrix of intact dentin is accessible for chemical reactivity or degradation by lytic enzymes is of importance in determining whether or not this portion of the tissue is the one initially attacked in dentinal caries. If it is not accessible for degradation by known lytic enzymes, then it is not likely that the bacteria producing these enzymes are a factor in the initiation of caries in dentin. However, as early as the turn of the century it was shown that proteolytic bacteria could lyse the organic matrix of dentin after its decalcification by various acids, or after decalcification in the early stages of natural caries. If the organic matrix of intact dentin is not accessible for lysis but becomes accessible during decalcification or during natural caries, the question then arises, when does it become available and what makes it available?

In an attempt to answer this question, we have measured the accessibility of certain chemical groups within the organic matrix of dentin that are indicative of a protein that can be lysed by certain proteolytic enzymes. The process of diazotization, which was utilized, is a specific measure of the accessibility of tyrosine and histidine in a protein. By partition chromatography, the presence of tyrosine and histidine in the organic matrix of dentin has been demonstrated by Atkinson and Matthews.[22] Bergmann and Fruton[19] have shown the essentiality of accessible tyrosine adjacent to the dipeptide linkage in the protein chain if such bonds are to be broken by pepsin or pepsin-like enzymes, or by carboxypeptidases. Since the ability of a protein to react with a diazohydroxide is indicative of the presence of accessible tyrosine, it is in turn indicative of the presence of a chemical configuration that could be broken by certain lytic enzymes. The application of the diazotization process

to determine the accessibility of the organic matrix of dentin has revealed something of the state of this portion of the tissue under varying conditions.

Dentin, completely decalcified by acids, not only reacted readily with the diazohydroxide but formed a constant amount of color per milligram of residual protein. Naturally carious dentin at the decalcification stage also reacted readily to form a constant amount of azoprotein per milligram of residual material. However, the amount of color formed per milligram by the naturally carious dentin was only approximately half that formed by the acid decalcified dentin (23.3 as compared to 50.0). Why this should occur is not known, though it is indicative that decalcification is not the only factor active in the carious process in dentin, for if it were, why did not both types of dentin react similarly?

In contrast, the organic matrix of intact, untreated dentin does not react readily with the diazohydroxide and neither is it readily accessible for lysis by ordinary proteolytic enzymes. The slight amount of diazotization that did occur in these sections was apparently referable to the protein along the walls of the pulp canal and in the dentinal tubules.

Several attempts were made to render the organic matrix of dentin accessible by exposing it to moist heat at various temperatures. The exposure of intact dentin to moist heat, for 60 minutes at 100° C., caused a slight decrease, rather than an increase, in the amount of accessible protein, but no other change. This reaction is in contrast to the solution of a major portion of the organic matrix of decalcified dentin by similar treatment. The organic matrix of intact dentin, exposed to moist heat for 30 minutes at 120° C., was slightly more accessible than intact dentin or dentin exposed to moist heat at 100° C.

The partial decalcification of dentin by nonequimolar and equimolar acids at a constant pH showed that its organic matrix became accessible for diazotization in proportion to the amount of weight lost. Therefore, it seems reasonable to conclude that the organic matrix of dentin is rendered accessible for reactivity with lytic enzymes by the action of acids. However, there are several possible ways by which this could take place, none of which can be completely substantiated by the present study. The first possibility is that the organic matrix is made physically accessible by removal of the inorganic ground substance during decalcification. This possibility is discounted somewhat by the fact that the surface of the organic matrix of cut dentin sections does not react with diazohydroxide. The second possibility is that the organic matrix is made accessible by the breaking of some bond that may exist between it and the inorganic portions. At the present time, we can do no more than speculate on this possibility. The third way in which the organic matrix may be made accessible is through a spacial rearrangement of the protein, or its degradation in some way by exposure to acids.

While the nonequimolar and equimolar acids rendered the organic matrix of dentin accessible in proportion to the amount of decalcification, statistical analyses of the data revealed several additional facts about its accessibility.

When different teeth were decalcified by nonequimolar acids at a constant pH there was, in six of ten comparisons, no significant difference between individual teeth in the amount of protein rendered accessible per unit of weight lost (Table V). On the other hand, when dentin was exposed to equimolar acids at a constant pH, the amount of protein rendered accessible per unit of weight lost varied between the individual teeth, except in three of ten comparisons (Table VIII). Why there is a difference in the amount of protein rendered accessible from different teeth under similar conditions is a matter of speculation. Several variables exist in the experiments, none of which offers an adequate explanation. The exact composition of the organic matrix of the different teeth could vary, which would lead to differences in the number of reactive groups in the decalcified protein. The possibility of existence of this difference is discounted somewhat by the constancy of the response of the teeth to nonequimolar acids at a constant pH. Another variable is the anionic concentration of the different types of acid solutions. This variation in anionic concentration is greatest, however, when the response of the organic matrix is most constant, i.e., when dentin is exposed to nonequimolar acids at a constant pH. Different types of anions were present but they were the same in each experiment.

An analysis of data on the rate of decalcification by the different types of acid showed that, in most instances, the different acids decalcified at different rates either when in nonequimolar or equimolar solutions, but at a constant pH. Since the hydrogen ion was constant in all solutions, the variability of the anion presumably accounted, in part, for the variability in the rates of decalcification.

CONCLUSIONS

Decalcification by various organic and inorganic acids liberated the organic matrix of dentin in proportion to the degree of decalcification (weight lost). The organic matrix of dentin rendered accessible by complete acid decalcification showed a constant amount of accessible protein per unit of weight, but this amount was twice that present in the organic matrix of naturally carious dentin at the decalcification stage. Partial decalcification of dentin by various nonequimolar acids at a constant pH liberated the organic matrix in a constant amount per unit of decalcification. On the other hand, the equimolar acids at the same pH rendered the organic matrix of different teeth accessible in significantly different amounts per unit of decalcification. At a constant pH, most of the organic and inorganic acids used to decalcify dentin did so at different rates whether the acid solution was nonequimolar or equimolar.

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DENTINAL PROTEIN: AMINO ACID COMPOSITION

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LOSEE, Neidig, and Hess[1] determined 13 amino acids in dentinal protein isolated by the method of Losee, Leopold, and Hess[2] and concluded from the results that dentinal protein resembled collagen. The three basic amino acids, arginine, histidine, and lysine, were determined by both colorimetric and microbiologic methods and the remaining 10 amino acids were determined by colorimetric methods only. We have now determined the six remaining amino acids and also repeated the determination of some of the amino acids previously estimated colorimetrically using microbiologic methods. Since no direct method of estimation of hydroxylysine could be found this amino acid was determined by the difference between the total hydroxyamino acids found by the periodic method previously employed[1] and the sum of the serine and threonine found microbiologically. The values obtained for the 19 amino acids account for 98.8 per cent of the total nitrogen in the protein and thus completely establish its amino acid composition.

EXPERIMENTAL

The media and general methods of analysis used by Horn, Jones, and Blum[3] were used for all the amino acids. However, for the preparation of the inoculums the media of Hac, Snell, and Williams[4] were used since we were unable to obtain the liver infusion employed by Horn, Jones and Blum.[3] *Leuconostoc mesenteroides* was used for the estimation of leucine and isoleucine and *Streptococcus faecalis* for the estimation of threonine and valine as recommended by them. The former microorganism was also employed for the estimation of leucine, isoleucine, valine, glutamic acid, aspartic acid, proline, glycine, and tyrosine and the latter microorganism was used for serine determination according to the recommendations of Neuman.[5] For each amino acid from three to four concentration ranges and triplicate determinations for each concentration were made and the results averaged. Turbidimetric procedures were discarded because the results on known concentrations were erratic and titration of the resulting lactic acid using 0.05N sodium hydroxide and bromthymol blue as the indicator was employed with completely satisfactory results.

The dentinal protein, prepared by the method of Losee, Leopold and Hess,[2] contained 16.15 per cent nitrogen. It was hydrolyzed with 20 per cent hydrochloric acid for six hours at 125° C. and the hydrolysate freed from acid by

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evaporation under reduced pressure. The results of these analyses and the earlier values are given in Table I together with values obtained by Neuman[5] on various collagens. It is apparent that the pattern of amino acid concentration in the dentinal protein falls well within that of collagen obtained from other

TABLE I. AMINO ACID CONTENT OF DENTINAL PROTEIN

	AMINO ACID (%)	AMINO ACID NITROGEN (%)	COLLAGENS AMINO ACID NEUMAN[5] (%)
Lysine	3.34*	0.640	5.9 - 5.7
Arginine	7.40[1]	2.380	8.6 - 9.4
Histidine	0.80[1]	0.216	0.56- 0.94
Hydroxyproline	13.10[1]	1.399	
Methionine	0.50[1]	0.047	0.69- 2.3
Phenylalanine	1.90[1]	0.161	2.3 - 2.6
Cystine	0.10[1]	0.011	0.05- 0.12
Tryptophane	0.0	0.0	0.0
Serine	3.50(3.10*)	0.466	2.8 - 3.2
Threonine	3.10(2.90*)	0.364	
Glycine	22.50(19.0*)	4.198	23.3 -29
Alanine	8.50	1.336	8.7 -10
Tyrosine	1.10(1.10[1])	0.085	0.86- 1.06
Proline	16.25	1.976	13.3 -16.5
Aspartic	5.63	0.592	6.7 - 7.5
Glutamic	10.62	1.011	10.8 -12.2
Valine	3.00	0.358	2.3 - 3.0
Isoleucine	1.25	0.133	1.5 - 2.2
Leucine	3.60	0.384	3.0 - 3.7
Hydroxylysine	1.00	0.184	
Total		15.941	(found 16.15)

*Previous values from Losee, Neidig and Hess.

sources. It can, therefore, be stated that dentinal protein obtained from normal human molars is a collagen, a finding confirmed by the recent electron microscopic studies of Scott, Albright, Sognnaes, and Wycoff.[6]

SUMMARY

Twenty amino acids have been determined in dentinal protein obtained from normal human molars accounting for 98.8 per cent of the total nitrogen. From the concentrations found of these amino acids it can be concluded that dentinal protein is a collagen.

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THE ISOLATION OF CHONDROITIN SULFURIC ACID FROM DENTIN

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A SUBSTANCE resembling chondroitin sulfuric acid by qualitative tests was first extracted by Pincus[1] from defatted human dentin with 10 per cent calcium chloride solution according to the method of Meyer and Smyth.[2] Rogers[3] estimated the amount of hexosamine in dentin by the Elson and Morgan[4] technique, and from this value calculated the amount of mucopolysaccharide present in the dentin to be 0.1 per cent. Subsequently Pincus[5] reported the isolation of 26.4 mg. of chondroitin sulfuric acid from one gram of dentin, an amount much greater than that found by Rogers.[3] The analytical data given by Pincus[5] do not conform to that expected for chondroitin sulfuric acid, particularly the high values for carbon, hydrogen, and nitrogen which range from 140 to 210 per cent of the theoretical. It is difficult to evaluate the findings of Pincus in the light of his conflicting analytical results. More recently Stack,[6] using the same method of determination as Rogers,[3] has reported 0.2 per cent of mucopolysaccharide in dentin.

In none of the reports cited were details given regarding the analytical procedures employed nor identification of the component amino sugar or uronic acid attempted. We have isolated chondroitin sulfuric acid from human dentin prepared from normal molars, and are giving the details of the procedure employed and also the evidence for the presence in the isolated material of galactosamine, the characteristic amino sugar of chondroitin sulfuric acid isolated from cartilage.

EXPERIMENTAL

Isolation of Chondroitin Sulfuric Acid From Bovine Tracheae.—In order to have a sample of chondroitin sulfuric acid as a reference material it was isolated as the potassium salt from bovine tracheae by the procedure of Einbinder and Schubert.[7] Bovine tracheae were obtained immediately after slaughter and, after mincing, were immersed in acetone. The material was dehydrated and defatted by several changes of acetone, at least twenty-four hours contact was allowed for each of three changes of the solvent. The weight of the air-dried material was 30 Gm. It was powdered and suspended in 500 ml. of an aqueous solution containing 20 per cent of KCl and 1 per cent of K_2CO_3 and shaken continuously for two days. The liquid was removed and the residue extracted with an additional 500 ml. of the same solution for an additional twenty-four hours. The supernatant liquids were combined and the residue

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saved for further extraction as detailed below. To the liquid portion were added 10 ml. of glacial acetic acid and 10 Gm. of potassium acetate, and, after the further addition of 10 Gm. of kaolin, the suspension was stirred for thirty minutes. After the kaolin was removed by filtration, the clear filtrate was dialyzed against distilled water for three days. The liquid within the dialysis bag was removed, and to it were again added 10 ml. of acetic acid, 10 Gm. of potassium acetate, and 10 Gm. of kaolin, and stirred for thirty minutes. After filtration the colorless filtrate was evaporated, under reduced pressure, to a volume of 100 ml., and after the addition of 5 Gm. of potassium sulfate it was poured into 500 ml. of absolute ethyl alcohol. A white precipitate formed immediately, the material was allowed to stand for twenty-four hours, and was then removed by centrifugation, washed with absolute alcohol and absolute ether, and dried in vacuo. The yield was 1.2 Gm. The solid residue, referred to previously, was suspended in 100 ml. of 2 per cent KOH solution and placed in a refrigerator for two days. The liquid portion was removed by centrifugation and treated in the same manner as the supernatant liquid from the original extraction. The yield was 2.5 Gm., and the total yield was 3.7 Gm. Analytical data on the potassium salt of the chondroitin sulfuric acid obtained are given in Table I.

Isolation of Chondroitin Sulfuric Acid From Dentin by the Einbinder and Schubert[7] *Procedure.*—One gram of dentin powder from normal human molars prepared as described by Losee, Neidig, and Hess[8] was extracted with 100 ml. of the potassium chloride—potassium carbonate solution as detailed previously. The entire procedure was employed as with the bovine tracheae, and the yield of the potassium salt of the chondroitin sulfuric acid was 6.4 mg. Analytical data on the preparation are given in Table I.

TABLE I. ANALYTICAL DATA ON CHONDROITIN SULFURIC ACID SAMPLES

SAMPLE SOURCE	HEXOSAMINE (%)	HEXURONIC ACID (%)	NITROGEN (%)
Bovine tracheae	28.07	29.20	2.13
Dentin*	27.78	25.08	2.63
Dentin†	15.3	14.0	
Bovine tracheae‡	23.55		2.15
Theory for $C_{14}H_{19}NSO_{14}\cdot 4H_2O$	29.49	31.96	2.31

*Preparation according to Einbinder and Schubert.[7]
†Preparation according to Pincus.[5]
‡Data from Einbinder and Schubert.[7]

Isolation of Chondroitin Sulfuric Acid by the Method of Pincus.[5]—One gram of dentin powder was dialyzed against 0.05N HCl for ten days, with frequent changes of the acid. The solution was brought to pH 6.0 and heated to 80° C. for twenty minutes. After cooling, 2 Gm. of solid $CaCl_2$ were added and the solution was dialyzed against distilled water for several days. The pH was again adjusted to 6.0, and the protein was removed by shaking with a mixture of 80 ml. of chloroform and 32 ml. of amyl alcohol per liter of solution. The aqueous layer was concentrated under reduced pressure and finally evaporated to dryness over P_2O_5 in a vacuum desiccator. The yield was 7.5 mg., and the analytical data are given in Table I.

Determination of Hexosamine.—A colorimetric method for the determination of hexosamine was first developed by Elson and Morgan[4] and modified slightly by Einbinder and Schubert.[7] In our hands the original Elson and Morgan method gave consistent results only with pure hexosamine and in rather concentrated solutions. The modification of Einbinder and Schubert was readily adapted for use on our materials, provided the acidity of the test solution was carefully controlled. We made a series of tests upon the color development using acidities from 0.2N to 7N HCl in the final test solution prior to color development, and found that the color varied from bluish purple at the highest degree of acidity to the desired pink at 0.2N HCl. Consequently we hydrolyzed the sample in a sealed tube with 2 ml. of 4N HCl by immersing the tube in a boiling water bath for ten hours. The hydrolysate was diluted with water to a volume of 50 ml., shaken with a small amount of decolorizing carbon, and filtered. This procedure gave the desired degree of acidity, and the color was then developed with the Einbinder and Schubert technique. The analytical results are included in Table I.

Determination of Hexuronic Acid.—The method of Dische[9] was applied to the various samples using glucuronic acid as a standard. Although Dische claimed a high degree of specificity for the carbazole reaction with hexuronic acids, he recognized the possibility of color formation by amino sugars or even simple hexoses. We found that fairly high concentrations of galactosamine gave a light greenish color. Glucose, lactose, and fructose were also tested, and all except glucose gave colors too weak to read. The amount of color produced by an equivalent weight of glucose was less than one-fourth that produced by glucuronic acid. From these results it is safe to conclude that we are actually determining hexouronic acid. The analytical data obtained with the method are included in Table I.

Determination of Hexuronic Acid and Galactosamine in Dentin Powder.—Applying the methods for the determination of these two components of chondroitin sulfuric acid indicated a galactosamine content equivalent to 0.39 per cent chondroitin and a hexuronic acid content equivalent to 0.27 per cent chondroitin in the original dentin. These values are of the same order of magnitude as those reported by Rogers[3] and Stack[6] using similar methods, but are actually less than the 0.64 per cent isolated as the potassium salt by the method of Einbinder and Schubert. The discrepancy is due to the losses encountered during the hydrolysis of the intact dentin, it was impossible to secure quantitative recovery with either glycuronic acid or glucosamine added to intact dentin.

Identification of the Amino Sugar Present in Dentin Chondroitin Sulfuric Acid by Paper Chromatography.—Chromatographic methods have been introduced recently for the separation of amino sugars as their dinitrophenyl derivatives. Anniton, James, and Morgan[10] employed partition chromatography with Celite as the stationary phase and a borate, amyl alcohol, chloroform solvent, carrying out the separation at 0° C., since decomposition occured at room temperature. Kent, Lawson, and Senior[11] used paper chromatography with a solvent composed of 40 per cent butanol, 10 per cent ethanol, and 50 per cent water. The solvent was allowed to flow for twenty-four hours, the paper strips

dried at room temperature and sprayed with the aniline phthalate reagent used by Partridge.[12] The paper chromatographic procedure appeared to offer less difficulty of manipulation, and the dinitrophenyl derivatives of glucosamine and of the hydrolyzed chondroitin sulfuric acid from bovine tracheae and dentin were prepared by the method of Anniton, James, and Morgan.[10] The separate Rf values for the three compounds were glucosamine 0.84, hexosamine from bovine tracheae 0.71, and the hexosamine from the dentin preparation 0.71. When the dinitrophenyl derivatives of glucosamine and dentin hexosamine were combined and chromatographed, excellent separation of the two spots was obtained. It is evident from these results that glucosamine is not present in dentin chondroitin sulfuric acid but that it and bovine tracheae chondroitin contain galactosamine, since they show an Rf value of 0.71, the same value reported by Kent, Lawson, and Senior[11] for this amino sugar.

DISCUSSION

The isolation of chondroitin sulfuric acid as its potassium salt from dentin by the method of Einbinder and Schubert[7] was effected with a yield of 0.64 per cent, a value far less than the 2.6 per cent reported by Pincus.[5] Using the method of Pincus, we obtained 0.7 per cent of a material that analytically was quite impure. As we have already noted, the analytical data published by Pincus do not agree with the theoretical values for chondroitin sulfuric acid, and we can draw no conclusions concerning the nature of his material. In particular, the nitrogen value obtained by Pincus of 5.4 per cent is almost double that required by theory; it is impossible to reconcile this value with the exactly theoretical acetyl value of 8.1 per cent he obtained. His values for hexosamine and hexuronic again are somewhat low, but not as low as they should have been in view of the high nitrogen value.

The analytical results for our preparation indicate a molecular ratio of hexosamine: hexuronic acid: nitrogen of 1:0.96:1.21, which is in close agreement with the theoretical ratio of 1:1:1. It will be noted also that our values for hexosamine are closer to the theoretical than that of Einbinder and Schubert for bovine tracheae chondroitin sulfuric acid. All isolation procedures are known to give rather poor yields, and it is quite probable that the actual amount of chondroitin sulfuric acid in dentin is at least two or three times as great as we have found.

Data based upon the hexosamine and hexuronic acid determinations upon intact dentin are unsatisfactory, since these compounds are readily destroyed by the acid hydrolysis required in the presence of the large amounts of inorganic salts. Our results by this procedure are in agreement with the results of Rogers[3] and Stack,[6] approximately 0.3 per cent chondroitin sulfuric acid, which is less than the amount isolated. Before we will have definite information concerning the actual amount present in dentin, improvements in methods of isolation and analysis will be necessary. However, we have shown that dentin does contain a chondroitin sulfuric acid analytically similar to that isolated from cartilage.

CONCLUSIONS

Chondroitin sulfuric acid has been isolated as its potassium salt from normal human dentin by the method applied to cartilage by Einbinder and Schubert,[7] with a yield of 0.64 per cent. Hexosamine and hexuronic acid determinations upon intact dentin gave a value of approximately 0.3 per cent, however, the analytical procedures for these compounds give low results due to destruction by the acid hydrolysis in the presence of large amounts of inorganic salts. The presence of galactosamine in the chondroitin sulfuric acid was shown by means of paper chromatography, indicating its similarity to chondroitin isolated from cartilage.

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EXPERIMENTAL DENTAL CARIES

I. The Effect of Orchiectomy and Ovariectomy on Dental Caries in Immature Rats

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EVIDENCE from the work of independent investigators indicates that gonadectomizing hamsters[1, 2] and rats[3] reduces the incidence of dental decay in immature males, while only a "biological" reduction appears in the females. Keyes[4] attempted to clarify this relationship by comparing the caries in a group of male and female gonadectomized hamsters with a similar group receiving testosterone. Under the conditions of his experiment, the data indicated that castrated males had slightly less caries than the noncastrated controls, while the castrated males receiving testosterone had a higher incidence than both former groups. In the females the controls and castrates had approximately the same amount of caries, while the castrates receiving testosterone had considerably less decay.

Previous work in this laboratory, using a strain of rats with a low degree of caries incidence,[5, 6] indicated that there appeared to be a difference in the incidence of caries in control male and female rats receiving the same diet. In the hope of clarifying this complex relationship between the sex of the animal and dental caries, this present study was designed to investigate whether the caries scores of castrated males would simulate the scores of uncastrated females, and if castrated female scores would resemble uncastrated males, as well as to determine whether the degree of caries would essentially return to its normal pattern in castrated animals by the administration of male or female sex hormones. A control group receiving the same concentration of sex hormones as the castrated group was tested on the basis that if removing the hormones would reduce caries, then increasing the hormone level might increase caries.

EXPERIMENTAL

A total of 134 animals,* of which 117 completed the experimental period, were divided as equally as possible as to sex, weight, and littermates into 8 groups. Each sex contained a control and a castrate group, and each control and castrate group was subdivided so that half received their respective sex hormones. The males received testosterone by implanting, subcutaneously, pellets weighing approximately 37 mg. between the scapulas. After 45 days pellets weighing approximately 75 mg. were implanted under similar conditions and those portions of these pellets remaining at the termination of the experiment

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*Carl Wilson strain, Beech Grove, Indianapolis, Indiana.

were removed and weighed in order that the dosage could be calculated. The females received 10 mg. of diethylstilbestrol in oil per week by subcutaneous injection.* All the animals were started on the experiment at 30 days of age and the investigation lasted for 117 days. All the animals received the caries-producing diet[5] and a fluoride-low water (0.1 μg per ml.) from birth. The method of preparing the heads for examination was reported previously.[5]

In the method for scoring caries, the extent or size of the various lesions was evaluated arbitrarily. For this purpose 3 different categories were recognized. A Number 1 classification was used for a very small cavity not penetrating the enamel, or for a superficial lesion on an exposed occlusal dentin surface. The use of the Number 2 designation was reserved for lesions which definitely extended into the dentin through the apex of the enamel-covered fissure and involved a relatively large area, but without extending beyond its own fissure or cusp. A Number 3 classification was used when the carious process had entirely destroyed its cusp or fissure and pulpal involvement was apparent. In the use of this system of classification the extent index for each animal was computed by determining the sum of the extent numbers and then taking the mean of those values. The mean value constituted the extent index for the series.

The expression "severity incidence" (SI) is a new method† for evaluating both the number of the lesions and the size of each lesion by one number. This figure is obtained by assigning a value of 1 to a tooth if less than one-fourth of it is destroyed by decay; a value of 2 if from one-fourth to one-half is destroyed; and a value of 3 if more than half is decayed. The arithmetic mean for each group is thus calculated.

At the end of the experimental period the animals were sacrificed and the heads removed in order to examine the teeth for caries, and the data are reported in this paper. In addition, the femurs were removed for breaking strength determination, calcium, phosphorus and fluorine determinations. The salivary glands were fixed by placing them immediately in Zenker's solution. The tongue, adrenal glands, and a section of long bones also were removed, as well as the lower jaw of each animal after caries had been scored, for histological examination. These data will be reported at a later date.

RESULTS AND DISCUSSION

The data for this experiment are presented in Table I. This table indicates the various experimental groups, the number of animals, and the number of cavities in each group. The extent of the lesions, the severity incidence (SI) and molars affected in each group are also shown. The data indicate that the number of lesions in the castrated males is considerably less than in the control males. Statistically, this difference is significant. The values for S.I. and extent of the lesions in this group further bear this out. The castrated males receiving testosterone appear to have fewer cavities than the control group, but the values for S.I. and extent of the lesions indicate that there is little difference between

*The Schering Corp., Bloomfield, N. J., and Mattox and Moore, Indianapolis, Ind., generously supplied the hormones used in this study.

†The authors wish to thank Dr. Arthur W. Radike for developing this method of counting caries.

these two groups. The control males receiving testosterone have a greater degree of caries than any of the other three groups. The difference between this group and the control group is not significant statistically, but the extent, S.I. and M.A. all indicate that some "biological factor" appears significant in this group.

The data for the females are quite different. The ovariectomized females have an increase in caries as compared to the control females. This is indicated by the number of cavities, the extent of the lesions, and severity incidence and molars affected, although statistically the data do not indicate significance. While the control males receiving testosterone had an increase in caries, the

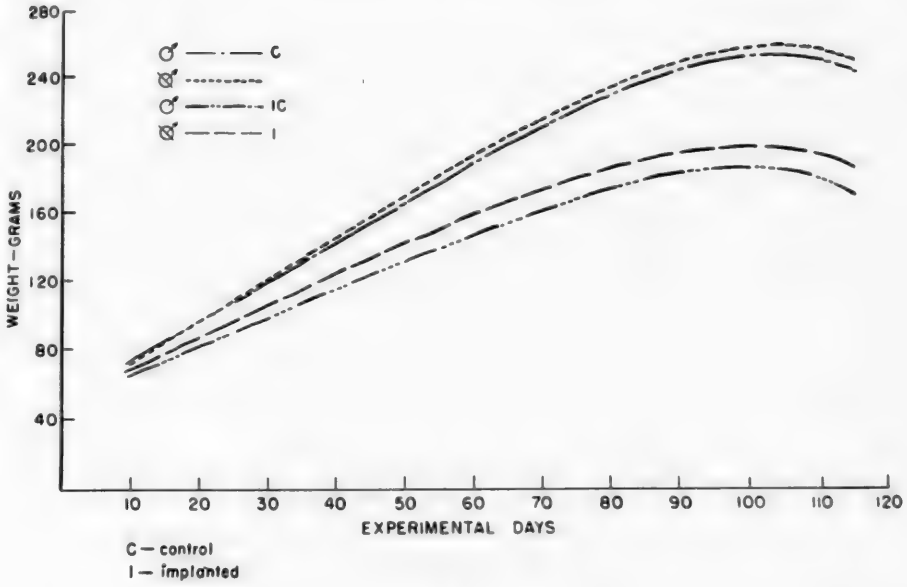
TABLE I

THE EFFECT OF ORCHIECTOMY AND OVARIECTOMY AND ADMINISTRATION OF TESTOSTERONE AND DIETHYLSTILBESTROL ON THE INCIDENCE OF DENTAL CARIES IN RATS RECEIVING A CARIES-PRODUCING DIET

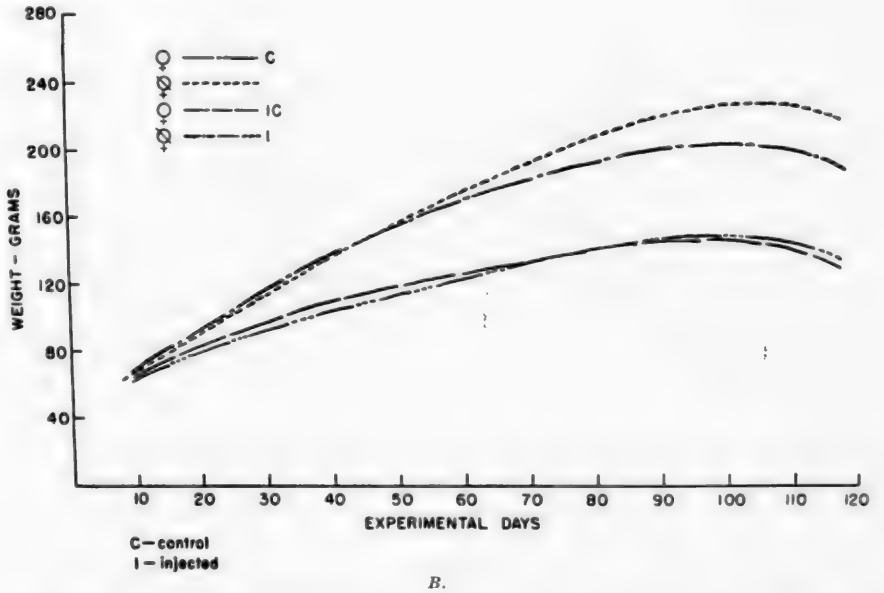
EXPERIMENTAL GROUP	NO. OF ANIMALS	NO. OF LESIONS	STANDARD DEVIATION	STANDARD ERROR OF THE MEAN	EXTENT	S.I.	M.A.
			Males				
Control	17	8.0	±0.93	0.23	1.82	10.40	4.70
Control+ Hormone	13	8.9	±0.69	0.19	2.12	11.82	5.15
Castrate	17	6.5	±1.28	0.31	1.27	8.21	4.31
Castrate+ Hormone	12	7.4	±0.80	0.23	1.71	10.30	4.50
			Females				
Control	16	7.1	±0.92	0.23	1.55	9.30	4.60
Control+ Hormone	12	6.5	±0.81	0.23	1.32	8.50	4.50
Castrate	17	8.4	±0.75	0.18	2.21	10.01	4.70
Castrate+ Hormone	13	9.0	±0.86	0.39	2.14	11.50	5.30

female controls receiving diethylstilbestrol have fewer lesions, smaller extent values and a lower caries incidence and molars affected. Thus, the decay pattern for castrated males receiving testosterone indicates fewer cavities as compared to the controls, while in the females the opposite situation occurred, there being a significant increase in caries in this group when compared with the control females. These data for the males corroborate the work of Keyes[4] on the hamster, but fail to substantiate the data for the female. This fact further indicates an inherent difference between the hamster and the rat in relation to their cariogenic activity. Our data in this laboratory using the Carl Wilson strain of rat have consistently shown a greater degree of caries in the male, while another strain (McCollum) has consistently shown the reverse.[5, 6] It is interesting that in this study, using the Carl Wilson strain, the control males again show at least a biological increase over the control females.

Our basic assumption that if castration should lower the incidence of decay, then the addition of the hormone might increase decay, appears to be true in the male but not in the female. These data compared with the discrepancy in the male and female castrate groups indicate that a more detailed study should be made pertaining to the effect of various hormones under different experimental conditions in the male and female rat before any definite conclusions are drawn.



A.



B.

Fig. 1.—The weight curves of the experimental animals. *A* indicates the weight changes in the four groups of male rates, while *B* indicates the increase in weight in the female animals.

A short discussion relative to the weights of the animals in each group should be presented. The initial mean weight values for the 8 groups of animals showed, at the maximum, a 4 Gm. difference between any 2 groups. By the end of the experimental period, during which bi-weekly weighings were made, the folllowing observations were noted: (1) no significant difference between control and castrated males; (2) a slight increase in weight of castrated females as compared to control females; (3) a marked diminution in weight of both males and females given testosterone and diethylstilbestrol, respectively, as compared to control males and females; (4) the same marked diminution in weight in castrated males and females given their respective hormones. In that large doses of these hormones have been shown to close prematurely the epiphysial center of ossification, this might account for a portion of the weight loss occurring in these animals. These comparisons are easily seen in Fig. 1.

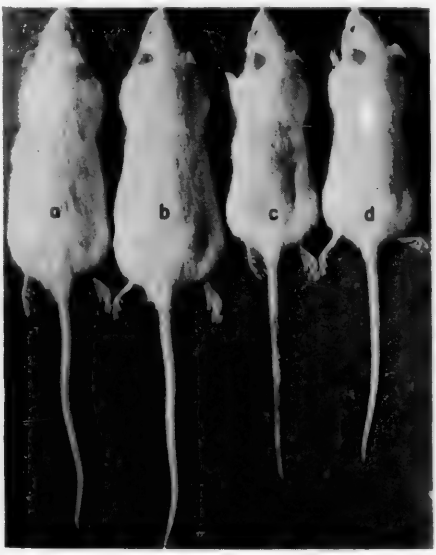


Fig. 2.—Comparison of females at termination of experimental period. *a* is control animal; *b*, castrate; *c*, castrate which has been injected with diethylstilbestrol; and *d*, control injected with diethylstilbestrol. Note loss of hair in animal *c*; skin in this group of animals is pigmented.

Figs. 2 and 3 demonstrate the gross weight changes indicated above, and in addition, show an additional experimental finding deserving some attention. In order to remove the ovaries, the hair on the dorsal surface of the back was removed. In those castrated females receiving the hormone the hair failed to return completely and, in addition, both the unoperated and castrated injected females had an extensive pigmentation of the skin. Similarly, in the castrated males the hair failed to grow back completely above the area operated upon for the implanting of the hormone, and the skin was also heavily pigmented. None

of the other animals presented this finding. Ginn and Volker[7] have indicated that rusting of the fur of the rat takes place in desalivated animals and correlate this finding with a faulty metabolism in some of the vitamins or sex hormones that were induced due to the removal of the salivary gland tissue.

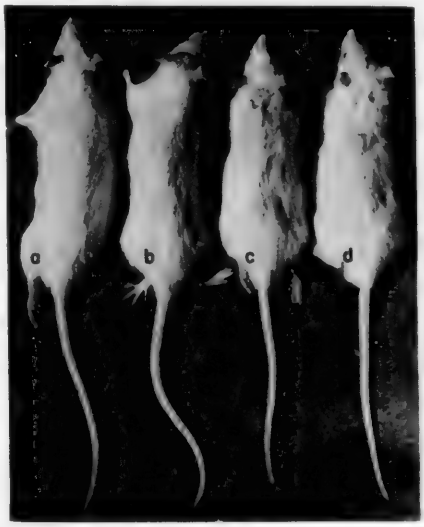


Fig. 3.—Comparison of males at termination of experimental period. *a* is control, *b* is castrate, *c* is castrate implanted with testosterone, and *d* is control implanted with testosterone. Note loss in hair in animal *c;* pigmentation of skin is marked in this group of animals.

CONCLUSIONS

1. The incidence of decay in immature orchiectomized rats is significantly less than control males, while ovariectomized rats show an increased amount of caries although this is not statistically significant.

2. The administration of testosterone to control males appears to increase decay, while the same hormone appears to reduce decay in gonadectomized males as compared to control rats. Conceivably, castration reduced the caries incidence in the male, but the hormone failed to return the decay incidence to normal.

3. The administration of diethylstilbestrol to control females appears to reduce the incidence of decay while similar treatment of ovariectomized animals significantly increases decay as compared to control animals.

4. Control females have less decay than control males, but the difference in the incidence of decay is not significant.

5. Failure to increase in weight in a normal manner was noted in both male and female castrate and control groups receiving testosterone and diethylstilbestrol, respectively.

6. Pigmentation of the skin and failure of hair growth on previously shaven areas were observed in castrated males and females receiving their respective hormones.

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THE EFFECT OF VITAMIN E DEFICIENCY ON THE RAT INCISOR

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ALTHOUGH the lack of pigmentation in the incisors of rats caused by vitamin E-deficient diet has been described frequently,[3, 4, 8, 12, 13, 14] reports on the histopathology of the enamel organ in this condition are scarce. In 1942, Irving[9] described premature and abnormal degeneration of the enamel organ in normally pigmented incisors of 3 rats which had been on a vitamin E-deficient diet for 167 days. Granados, Mason, and Dam[6, 7] reported progressive atrophy of the enamel organ and an increasing amount of acid-fast pigment in macrophages at the perivascular and fibrous zones surrounding the enamel organ. Their rats were fed vitamin E-deficient diets high in lard, cod liver oil, or in the highly unsaturated fatty acids of the latter. Using a purified diet containing 0.1 per cent succinylsulfathiazole and deficient in vitamin E, Pindborg[13] found premature and sudden atrophy of the papillary layer, marked edema, and cyst formation in the enamel organ.

The purpose of the present paper is to describe in detail the histologic changes caused by feeding different diets, all deficient in vitamin E.

MATERIAL AND METHODS

The rats used in the experiments were 1-month-old white rats of the Wistar strain. The animals were kept in individual cages and were weighed every week. The animals were divided into five groups (Table I):

Group A received the purified vitamin E-deficient diet (I) used in previous experiments.[13] The salt mixture used was McCollum No. 185. One hundred twenty rats received this diet in periods varying from 23 to 425 days.

Group B (control group) received the same diet supplemented with 3.5 mg. tocopherol daily per rat. Twenty-eight rats received this experimental diet in periods varying from 34 to 221 days.

Group C received the fat-free, vitamin E-deficient diet (IIa) used by Granados and Dam.[5] Ten rats received this diet in periods varying from 66 to 119 days.

Group D received the same diet as Group C but 20 per cent sucrose was replaced by 20 per cent cod liver oil (IIb). Fifteen rats received this diet in periods varying from 66 to 119 days.

Group E† received a diet made deficient in vitamin E by treating with ferric chloride according to Waddell and Steenbock.[15] Ten rats received this diet in periods varying from 3 to 10 months.

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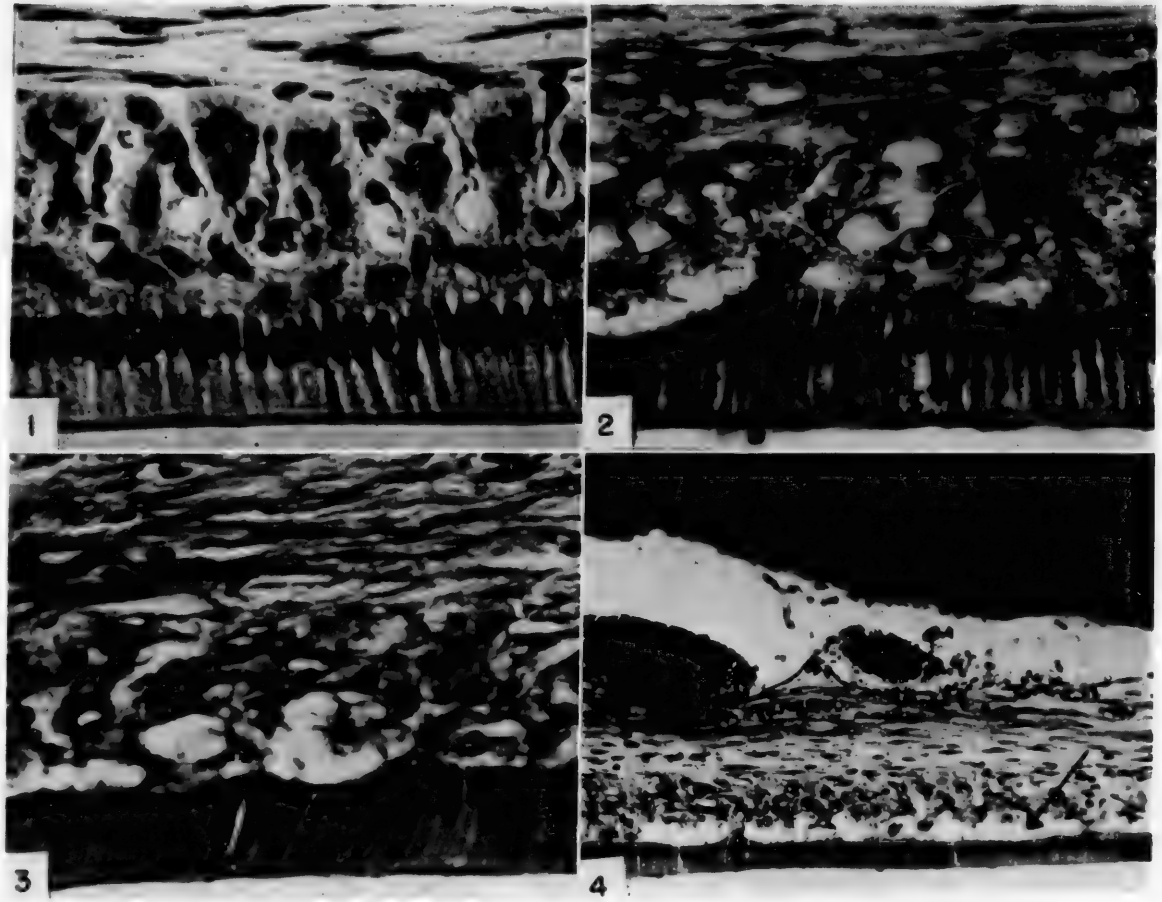
†The material used in this group was kindly furnished by Dr. F. B. Adamsstone, Dept. of Zoology, University of Illinois, Urbana, Ill.



Figs. 1 to 4.—Photomicrographs of the middle third of the enamel organ of upper incisors.

1, Control rat receiving stock diet. Note columnar ameloblasts and well-contoured papillary layer. Orig. mag. ×600.

2, Rat (No. 433) receiving vitamin E-deficient diet (Group A) for 213 days. Note edema and beginning disorganization of papillary layer. Orig. mag. ×600.

3, Rat (No. 432) receiving vitamin E-deficient diet (Group A) for 213 days. Note edema and advanced disintegration of papillary layer. Orig. mag. ×600.

4, Rat (No. 444) receiving vitamin E-deficient diet (Group A) for 213 days. Note extended edematous area in papillary layer as indicated by arrow. Orig. mag. ×200.

TABLE I

THE EFFECT ON THE UPPER RAT INCISOR OF DIFFERENT DIETS DEFICIENT IN VITAMIN E

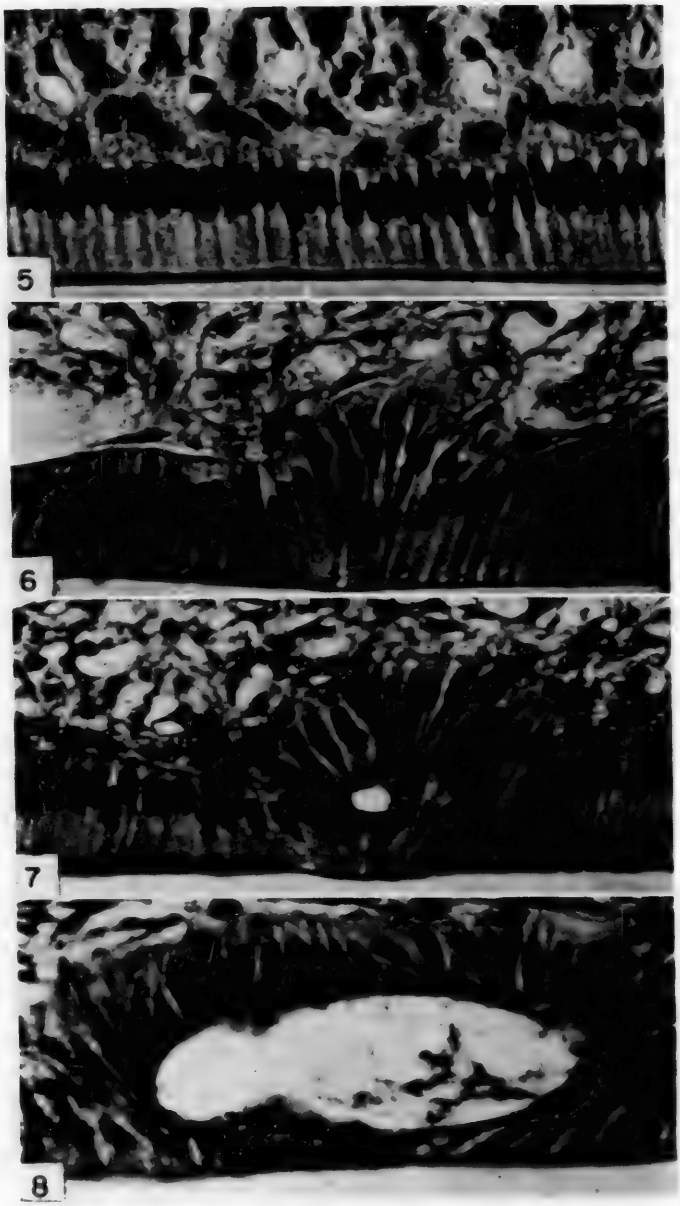
DIET	GROUP	NO. OF RATS	LACK OF PIGMENTATION	HISTOLOGIC CHANGES	EXPERIMENTAL PERIOD RANGE
Vitamin E-Deficient Diet I	A	120	+	+	33-425 Days
Same Diet Plus 3.5 mg. Tocopherol Daily	B	28	–	–	34-221 Days
Vitamin E-Deficient Diet IIa (Fat Free)	C	10	(+)	–	66-119 Days
Vitamin E-Deficient Diet IIb (Plus Fat)	D	15	+	+	66-119 Days
Vitamin E-Deficient Diet III	E	10	+	+	180-270 Days

After autopsy, the teeth were examined macroscopically for the degree of their pigmentation and then fixed in formalin. After decalcification in 5 per cent nitric acid and embedding in celloidin central sections of the upper incisors were stained with hematoxylin and eosin.

RESULTS

Pigmentation.—In 3 (A, D, and E) of the 4 groups fed a vitamin E-deficient diet, the lack of incisor pigmentation was almost entirely restricted to the upper incisors, though the degree of bleaching varied considerably. The first signs of pigment deficiency were seen about 50 days after the start of the experiment. The lower incisors in these groups showed only occasional slight change. In contradistinction, 5 animals of Group C showed involvement of the lower incisors 65 days after the start of the experiment. The lack of pigmentation in the lower incisors was only partial. All rats receiving tocopherol (Group B) were protected completely against changes in the pigmentation of the incisors.

Histology of the Normal Enamel Organ.—Before presenting the experimental findings, the histology of the enamel organ in the normal upper rat incisor shall be reviewed briefly. The labial odontogenic epithelium differentiates into the ameloblasts and the papillary layer. The latter is composed of the stratum intermedium and outer enamel epithelium of the enamel organ and includes numerous capillary invaginations from the surrounding connective tissue (Fig. 1). In the early stage of amelogenesis the papillary layer is low, while the ameloblasts are tall. Just before the enamel matrix becomes acid-soluble, the papillary layer gains in height and at a level corresponding to the middle of the alveolus it reaches the maximal height (Fig. 1). At the border between the middle and incisal third of the enamel organ the papillary layer



Figs. 5 to 8.—Photomicrographs of the middle third of the enamel organ of upper incisors. Orig. mag. ×600.

5, Control rat receiving stock diet, same as *Fig. 1*.

6, Rat (No. 437) receiving vitamin E-deficient diet (Group A) for 213 days. Note piling up of ameloblasts into edematous tissue.

7, Rat (No. 437) receiving vitamin E-deficient diet (Group A) for 213 days. Note that folding of ameloblasts has progressed.

8, Rat (No. 437) receiving vitamin E-deficient diet (Group A) for 213 days. Note cavity formed by folding ameloblasts.

and the ameloblasts begin to atrophy. When the crest of the labial alveolar bone is reached the enamel organ is further reduced in thickness. The incisors of rats in Group B showed normal structure of the enamel organ.

HISTOLOGIC CHANGES IN THE ENAMEL ORGAN OF THE UPPER INCISOR IN VITAMIN E DEFICIENCY*

Papillary Layer.—All animals of Groups A, D, and E showed typical changes in the papillary layer after about 50 days on the experimental diet. At a level corresponding to the completion of the enamel matrix the connective tissue papillae show the sign of beginning edema (Figs. 2 and 3). The connective tissue and epithelial cells in the attacked area appeared necrotic as indicated by pyknotic nuclei (Fig. 4). In most animals the disintegration of the papillary layer occurred abruptly. A gradual onset of the changes could be observed in some cases. The necrotic and edematous areas varied in size from small isolated islands to more extended areas (Fig. 4).

Ameloblast Layer.—The changes in the ameloblastic layer were changes in the timing of the reduction of ameloblasts or distortions of the normal arrangement of these cells. Normally, the reduction of the ameloblasts in height precedes the atrophy of the papillary layer. In cases of vitamin E deficiency, however, the atrophy of the ameloblasts occurs at an earlier level and is intimately associated with the changes of the capillaries in the papillary layer. In the advanced stages ameloblasts and the epithelial cells of the papillary layer are fused so that any distinction between the two layers is impossible.

In most animals cystic cavities (the development of which could be traced) form in the ameloblast layer. The first sign of this disorder is a folding of the ameloblastic layer in an area of edema. The ameloblasts are seen to pile up at this point, but do not lose their contact with the enamel cuticle (Fig. 6). While this fold increases in height, a cavity forms between shortened cells that adhere to the enamel and the longer cells that are folded away from it (Fig. 7). This minute cavity may increase to a considerable size. It is filled with a coagulated fluid containing some cellular debris (Fig. 8).

In some animals the ameloblast layer is detached from the enamel cuticle by an accumulation of fluid.

The upper incisors of the rats in Group C receiving the fat-free vitamin E-deficient diet did not show any deviation from the normal histologic structure in the enamel organ.

DISCUSSION

The present experiment confirmed previous reports concerning the effect of vitamin E deficiency on the enamel organ and the pigmentation of the rat incisor. In the present study the causes of the changes in the enamel organ could be traced to an edema of the papillary layer. This edema in turn is most probably caused by a primary damage of the capillary walls in the papillary layer.

*The bleached lower incisors of Group C have not been examined. Findings on the enamel organ of these teeth will be reported at a later date.

Besides other effects, vitamin E seems to play some role in vascular physiology and particularly in the maintenance of normal capillary permeability. Mason[11] found vascular changes consisting of vasodilation and congestion in rat fetuses from mothers fed a vitamin E-deficient diet. Also in birds capillary damage caused by vitamin E deficiency has been observed. Dam and Glavind[2] described a condition of exudative diathesis in chicks fed vitamin E-deficient diet, characterized by an accumulation of fluid in the subcutaneous connective tissue. This finding, confirmed by Bird and Culton,[1] has been ascribed to an increased permeability of capillaries.

The edematous infiltration of the connective tissue of the papillary layer induces a disintegration of the epithelial papillae and a subsequent folding of the ameloblasts in the area of mature enamel. This in turn leads to formation of cystic cavities. In no instance was a disturbance in the alignment of the ameloblasts seen without change in the papillary layer.

The folding and piling up of the amebloblasts into the edematous connective tissue can be explained by the fact that the incisors and the enamel organ are continuously growing and moving (erupting) tissues. Since in the area of mature enamel a hard and a soft tissue have to move in unison, the slightest interference may cause a dissociation of these tissues.

While observations on rats and chicks lend support to the assumption that vitamin E deficiency causes capillary damage in the enamel organ of the rat incisor, the question naturally arises why other organs or tissues in the rat do not show the same increase in permeability of the capillaries. An answer to this question may be found in the fact that the rat incisor and its enamel organ are continuously growing tissues and as such are more sensitive to systemic disturbances. It has been stressed by Mason[11] that in chicks the disturbance of the vascular system occurs only during the actively growing period of the bird. From other nutrition deficiencies it is known that the incisor is more sensitive than other organs or tissues of the rat organism (Irving and co-workers).[10]

SUMMARY

The present study is based on the gross anatomic and histologic investigation of the upper incisors of 185 rats kept on four different diets all deficient in vitamin E for 33 to 425 days. Rats fed on three of these diets showed lack of pigmentation of the upper incisors to a different degree.

The rats receiving a fat-free but vitamin E-deficient diet showed no changes in the pigmentation of the upper incisors and only occasionally changes in the lower incisors. Addition of tocopherol to the diet protected the animals from any symptoms of vitamin E deficiency.

The histologic changes in the enamel organ were the following: (1) Damage of the capillary walls in the papillary layer, (2) development of an edema in the papillary layer, (3) folding of the ameloblasts into the adjacent edematous connective tissue and formation of cysts, and (4) change in the timing of atrophy of papillary layer and ameloblasts.

The author wishes to thank Drs. I. Schour, H. Sicher, and J. P. Weinmann for their valuable criticism and help.

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THE REPARATIVE EFFECT OF ANTABUSE (TETRAETHYLTHIURAM DISULFIDE) UPON DENTAL CHANGES IN VITAMIN E DEFICIENT RATS

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INTRODUCTION

AFTER it was found that vitamin E deficiency causes lack of pigmentation of the rat incisors, some investigators tried to find substances other than tocopherol which would restore pigmentation or prevent lack of pigmentation. Successful results have been obtained by adding nordihydroguaiaretic acid, manganese, and methylene blue to the vitamin E deficient diet.[1, 3] Furthermore, Aaes-Jorgensen, Dam, and Granados[1] found that 0.025 per cent Antabuse (tetraethylthiuram disulfide) incorporated in a vitamin E deficient diet provided good protection against lack of pigmentation of the incisors.

This observation was confirmed using a different vitamin E deficient diet in the present series of experiments and was extended by a histologic examination of the enamel organ.

MATERIAL AND METHODS

This study is based on the gross anatomic and histologic examination of 20 white rats of the Wistar strain, 1 month of age. The animals were kept in individual cages and weighed every week.

The basal vitamin E deficient diet used was of the composition shown in Table I.

After 4 months on this diet the color of the upper incisors was recorded (3/8/51). Ten rats continued to receive the original diet for an additional 3 months (Group I). The remaining 10 rats received for the same period of 3 months 0.025 per cent Antabuse mixed into the experimental diet (Group II).

After 3 months, all the animals were sacrificed and the color of the upper incisors was recorded (6/16/51). The incisors were then decalcified, embedded in paraffin, sectioned, and stained with hematoxylin-eosin and van Gieson-Hansen's connective tissue stain.

FINDINGS

Weight.—The addition of Antabuse to the diet caused at first in all 10 rats a slight decrease in weight. However, after 4 weeks the Antabuse-treated rats gained more in weight than the rats given the basal diet.

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*Public Health Service Research Fellow of the National Institute of Dental Research.

TABLE I

COMPOSITION OF THE BASAL DIET

Glucose	70	Vitamin mixture:	
Casein, purified	20	Choline HCl	1 Gm.
Salt mixture (McCollum No. 185)	4	Nicotinic acid	50 mg.
Groundnut oil and 1600 I.U. Vitamin A and 150 I.U. and Vitamin D	3	Thiamine HCl	10 mg.
		Riboflavin	10 mg.
		Ca pantothenate	50 mg.
Vitamin mixture	2	Pyridoxine HCl	10 mg.
Succinylsulfathiazole	1	P-aminobenzoic acid	10 mg.
	100	Vitamin K	5 mg.
		Glucose to make	20 Gm.

Pigmentation.—Table II shows the changes in color of the incisors of the experimental rats. After 4 months on the vitamin E deficient diet all the rats, except one (No. 450), showed lack of pigmentation. The rats in Group I, continued on the original vitamin E deficient diet, exhibited after 3 more months an almost total lack of incisor pigmentation. When, however, Antabuse was added to the diet for 3 more months, the rats (Group II) showed complete restoration of the pigmentation of the incisors.

TABLE II

THE INFLUENCE OF VITAMIN E DEFICIENT DIETS WITH AND WITHOUT ADDITION OF ANTABUSE UPON THE COLOR AND HISTOLOGY OF THE UPPER RAT INCISOR

VITAMIN E DEFICIENT DIET				VITAMIN E DEFICIENT DIET PLUS 0.025% ANTABUSE			
	COLOR OF THE UPPER INCISORS*		HISTOLOGIC SYMPTOMS OF VITAMIN E DEFICIENCY		COLOR OF THE UPPER INCISORS*		HISTOLOGIC SYMPTOMS OF VITAMIN E DEFICIENCY
NO.	3/8/51	6/16/51		NO.	3/8/51	6/16/51	
432	3	2	+	434	6	10	–
433	3	3	+	438	3	9	–
435	2	2	+	439	4	9	–
436	2	3	+	440	3	10	–
437	3	1	+	441	4	10	–
442	5	3	+	446	6	10	–
443	7	1	+	447	4	10	–
444	6	3	+	448	6	10	–
445	4	1	+	449	5	10	–
450	10	2	+	451	9	10	–

*The color was graded after a scale where 10 represents the color of a normal upper incisor and 1 the complete lack of pigmentation.

Histology.—All the upper incisors from rats in Group I showed the changes typical for vitamin E deficiency[5]: damage of the capillary walls in the papillary layer, development of an edema in the papillary layer, and folding of the ameloblasts into the adjacent edematous connective tissue with formation of cysts. In contrast to this, none of the rats receiving Antabuse in the vitamin E deficient diet (Group II) showed any changes in their enamel organ.

DISCUSSION

From the present study and the report by Aaes-Jorgensen, Dam, and Granados, it appears that Antabuse restores the disturbances in pigmentation of the incisor in rats kept on a vitamin E deficient diet. Furthermore the

present histologic investigation indicated that the changes of the enamel organ typical for vitamin E deficiency are prevented by addition of Antabuse to the diet. In other words, Antabuse is able to replace vitamin E or tocopherol in preventing the changes of the rat incisor.

Rats kept on a vitamin E deficient diet containing highly unsaturated fatty acids develop a brown coloration of the adipose tissue which can be prevented by the addition of Antabuse to the diet. It has been shown that the changes of the enamel organ in vitamin E deficient rats are due to a preceding damage of the capillaries.[5] The ability of Antabuse to counteract a damaging effect upon the capillaries in rats has not previously been reported. However, in vitamin E deficient chicks, Antabuse affords a partial protection against an exudative diathesis due to increased capillary permeability.[2]

The mechanism by which Antabuse imitates the action of α-tocopheral is not clear. However, it is known that Antabuse has, similar to vitamin E, an antioxidant property. It is interesting that Antabuse, which is used as a drug producing hypersensitivity to alcohol,[4] is able to prevent some of the clinical and histologic symptoms of vitamin E deficiency.

SUMMARY

The present study is based on the anatomic and histologic examination of 20 rats. One-month-old rats were kept on a vitamin E deficient diet for a period of 3 months. Ten of the rats then received 0.025 per cent Antabuse in addition to the experimental diet for a period of 4 months, while the other 10 were continued on the experimental diet alone.

The rats receiving the vitamin E deficient diet showed lack of pigmentation and typical histologic changes in the enamel organ. Rats receiving Antabuse added to vitamin E deficient diet did not show any changes in the incisors.

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SYMPTOMS OF VITAMIN A AND E DEFICIENCIES IN THE INCISOR OF THE RAT FED SODIUM SULFITE

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FOR many years sulfites have been used for food preservation. During World War II when troops were serving in tropical climates it became necessary to sulfurize dried fruit to a much higher extent than heretofore, and the question of possible toxic effects became important.

Fitzhugh, Knudsen and Nelson[2] studied the effect on rats of diets containing sulfites. They observed polyneuritis, focal myocardial necrosis and fibrosis, which they interpreted as being caused by a thiamine deficiency. The animals furthermore developed "spectacle eyes," brown uteri, atrophy of various viscera, and changes in the kidneys and bones. The changes were complex and were discussed in some detail.

During the course of these experiments, it was noticed that the incisor teeth, normally orange in color, gradually became white. This decoloration is itself of considerable interest, because it has been shown that it can be produced to a varying degree by several different experimental procedures.

A further stimulus to the present investigation was the question whether the lack of pigmentation is accompanied by histologic changes of the incisor.

MATERIAL AND METHODS

This investigation is based on the gross anatomic, roentgenologic and histologic examination of the teeth and adjacent structures of 43 of the rats used by Fitzhugh, Knudsen and Nelson[2] (Table I). At 21 days of age, weaning time, these Osborne-Mendel rats had varying amounts of sodium bisulfite ($NaHSO_3$) added to a basic diet consisting of:

Cornstarch	60 per cent	Casein	18 per cent
Corn oil	6 per cent	Brewer's yeast	5 per cent
Powdered whole liver	5 per cent	Salt mixture (U.S.P. XII No. 2)	4 per cent
		Cod liver oil	2 per cent

Eight groups of 18 to 24 animals each received an addition of 0.0125, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0 and 1.5 per cent sodium bisulfite to the basic diet. After 3 months the concentration of sulfite for the last group was increased from 1.5 to 2 per cent. A similar group of animals fed only the basic diet was kept as controls. The dry sulfite powder was mixed with the basic ration by means of a dough mixed. The diets were refrigerated until used. All animals were kept in individual cages in a room with controlled temperature and humidity and

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were given free access to their respective diets and water. At weekly intervals body weights and food consumption were determined, and a supply of food sufficient to last a week was placed in each food cup. Surviving animals were sacrificed after 1 to 2 years. The heads of animals that were found dead or that were sacrificed at the end of the experiment were bisected and preserved in a 10 per cent formalin solution.

The experiment was carried out in the laboratories of the Food and Drug Administration in Washington. A selected group of 43 rat heads were sent to the Department of Histology, College of Dentistry, University of Illinois. The teeth were examined macroscopically for the degree of pigmentation. The skulls were x-rayed and were then decalcified and embedded in paraffin. Central sections of the incisor and molar teeth were stained with hematoxylin and eosin or impregnated with silver (Gomori).

FINDINGS

A. Pigmentation of the Incisors.—The normal enamel of the upper incisors of the Osborne-Mendel strain of rats is deep orange in color. After feeding sulfites there was a lack of pigmentation, the degree of which was generally speaking proportionate to the concentration of sulfite and to the duration of the experiment (Table I). However, upper and lower incisors were not always affected to the same degree. Thirteen rats showed no changes in the pigmentation and of the remaining 28 rats 20 had whiter upper incisors and normal lower incisors. Only 8 had both the upper and the lower incisors depigmented.

TABLE I

THE EFFECT OF SODIUM SULFITE ON THE UPPER INCISOR OF THE RAT

GROUP	CONCENTRATION OF SULFITE (%)	NUMBER OF ANIMALS	DURATION OF EXPERIMENT (YEARS)	NUMBER OF ANIMALS WITH: CHANGES IN INCISOR PIGMENTATION	PATHOLOGIC CHANGES IN ENAMEL ORGAN	SHORT ENAMEL MATRIX	CESSATION OF LINGUAL DENTIN FORMATION	INVAGINATION OF LINGUAL ODONTOGENIC EPITHELIUM	DISTORTED (FOLDED) ENAMEL MATRIX	KERATINIZATION IN NASOLACRYMAL DUCT	THICKENING OF FUNDIC ALVEOLAR BONE
O	–	2	1	0	0	0	0	0	0	0	0
C	0.0125	2	1½	2	2	0	0	0	0	0	0
A	0.025	6	1	1	0	0	0	0	0	(1)	0
B	0.05	5	1	2	1	0	0	0	0	0	0
D	0.1	5	1	1	2	1	0	1	0	0	1
E	0.25	3	1	0	1	1	0	0	0	(1)	0
F	0.25	2	1½	2	0	0	0	0	0	0	0
G	0.5	3	1	2	3	1	0	0	0	0	0
H	0.5	4	1½	4	4	1	2	2	1	0	1
K	1.0	3	1	3	3	1	3	3	2	3	3
L	1.0	3	More than a year	3	3	3	3	3	3	3	3
M	2.0	3	Less than a year	3	3	2	3	2	0	2	3
N	2.0	2	1	2	2	2	2	2	2	2	2

With increasing dosage the abrasion pattern especially of the lower incisors was irregular, and in several cases the upper incisors were fractured.

B. Histology of the Normal Rat Incisor.—The rat incisor is a continuously growing tooth that is covered only on the convex side with enamel, while its concave surface is covered by a thin layer of cementum. The incisors develop primarily from an epithelial base, the odontogenic epithelium. This epithelium behaves differently at the labial and the lingual side.

Lingually the epithelium consists of one to two rows of spindle-shaped cells, that are comparable to the cells of Hertwig's epithelial root sheath of teeth with limited growth. Like the latter the lingual epithelial cells have retained their qualities to induce differentiation of odontoblasts although they do not form enamel.

Labially the epithelium differentiates into the ameloblasts and the papillary layer. The latter is composed of the stratum intermedium, stellate reticulum, outer enamel epithelium of the enamel organ, and includes numerous capillary invaginations from the surrounding connective tissue (Fig. 1).

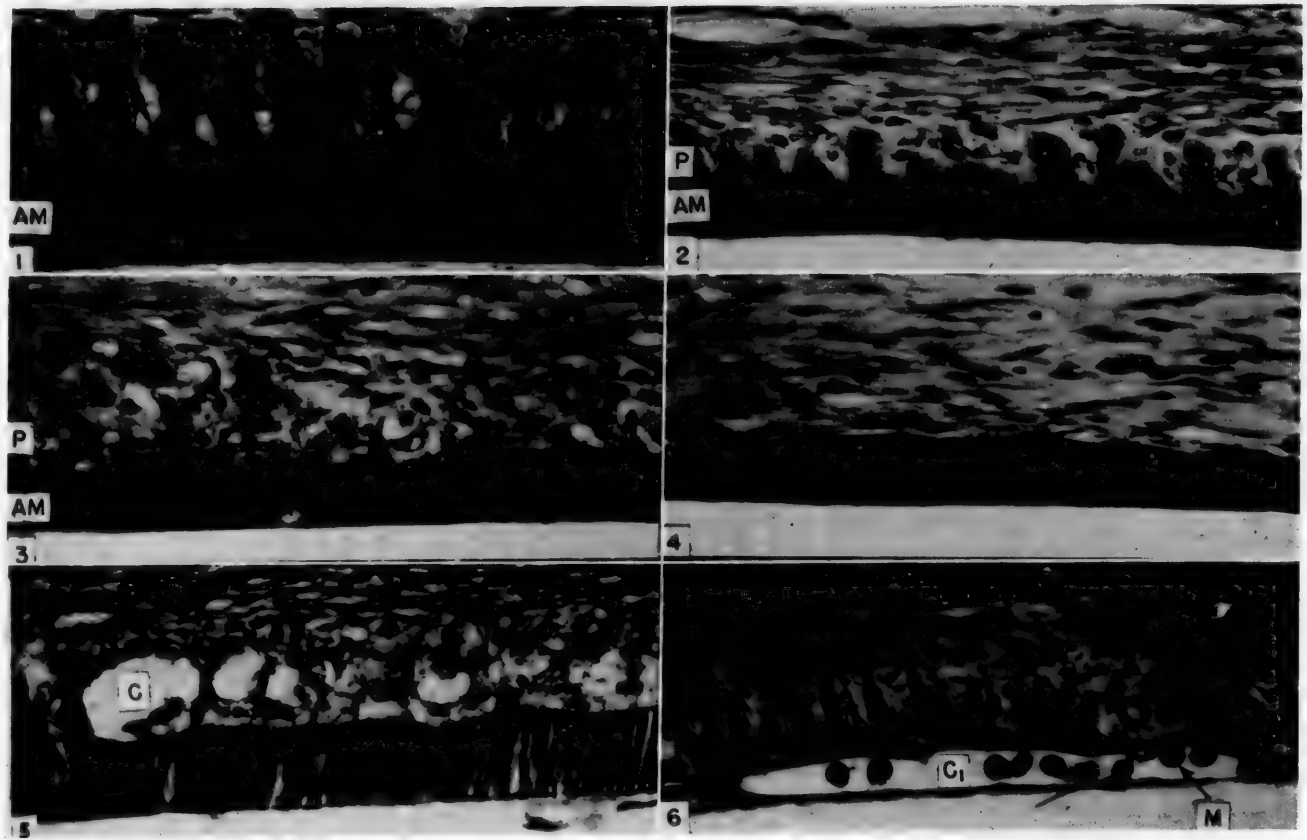
After forming the enamel matrix the ameloblast as well as the other layers of the enamel organ undergo a physiologic atrophy. In the normal adult rats of the Osborne-Mendel strain the atrophic changes can be observed about midway between the basal end of the tooth and the gingival crevice. In an incisal direction, the first sign of the atrophy is the progressive reduction of the height of the ameloblasts, that gradually assume a cuboidal shape (Fig. 2). The integrity of the papillary layer of the enamel organ is undisturbed in this area but farther incisally the folds of this layer gradually straighten out and from here on the enamel is covered by 5 to 6 layers of cuboidal to spindle-shaped epithelial cells.

The odontogenic epithelium establishes the dentino-enamel and dentino-cemental junctions and activates the adjacent cells of the pulp to differentiate into odontoblasts. Normally the widths of the labial and lingual dentin at the midlevel of the tooth are essentially equal. As a result of the continuous formation of dentin the pulp narrows toward the incisal edge.

The alveolar bone of the upper incisor consists of anterior and posterior portions which are separated by the premaxillo-maxillary suture. The posterior maxillary portion forms the fundus and the posterior third of the labial alveolar bone. The fundic bone is extremely thin and owing to its shift toward the tooth by sutural growth at the premaxillo-maxillary suture, osteoclastic resorption can be seen on the periodontal side.

C. Histologic Changes in the Incisors of Sulfite-fed Rats.

Enamel organ and enamel: In normal adult rats the enamel organ undergoes a physiologic atrophy at about the middle of the incisor. In the experimental rats the area of atrophic changes had shifted frequently to the basal third. The atrophy of the enamel organ is not only premature but also atypical. Normally the atrophy starts with a reduction of the ameloblasts in height and is followed by a reduction of the papillary layer. In the experimental rats the regression set in with a sudden reduction of the papillary layer (Fig. 3) and was then followed by atrophy of the ameloblasts. Furthermore, whereas physiologic atrophy progresses continuously and regularly until the reduced enamel



Figs. 1 to 6.—Photomicrographs of the enamel organ of the upper rat incisor. Hematoxylin-eosin stain. Orig. mag. ×400. AM = ameloblasts. P = papillary layer.

Fig. 1.—Middle third of normal incisor.

Fig. 2.—Incisal third of normal incisor. Note the shortened ameloblasts and the reduction of the papillary layer.

Fig. 3.—Middle third of incisor of rat fed 0.1 per cent sulfite for one year. Note the shortened ameloblasts and the atrophy of papillary layer in contrast to the tall ameloblasts and papillary layer in Fig. 1.

Fig. 4.—Incisal third of incisor of rat fed 0.1 per cent sulfite for one year. Note the absence of the papillary layer and ameloblasts, which have become replaced by densely and irregularly arranged polyhedral cells.

Fig. 5.—Middle third of incisor of rat fed 0.5 per cent sulfite for one year. Note edema and cavitation (*C*) in papillary layer.

Fig. 6.—Incisal third of incisor of rat fed 0.1 per cent sulfite for one year. Note cystlike cavity in reduced ameloblastic layer (*C*) with inclusions of macrophages (*M*) and the elongated and disturbed papillary layer.

organ fuses with the gingival epithelium, atrophy of the sulfite-fed rats is irregular and areas of far-advanced atrophy alternate with areas of a fairly normal enamel organ.

In advanced stages of atrophy the enamel organ consists of stratified epithelium, the single cells of which are of irregular shape and densely packed (Fig. 4). In the incisal third the atrophic enamel organ consists only of 1 to 2 layers of epithelial cells.

In some of the animals the picture of pathologic atrophy was complicated by different degrees of edema in the adjacent connective tissue leading to the formation of small rounded spaces between the cells of the papillary layer (Fig. 5). Often small cystlike cavities were formed (Fig. 6) in the fluid of which macrophages could be observed.

The experimental changes in the enamel organ had occurred after completion of enamel formation. It is therefore not surprising that as a rule the enamel was normal. Enamel hypoplasia was observed in only one instance. However, the length of the nonacidsoluble enamel matrix appeared reduced in some of the animals fed lower doses of sulfite and in most of the animals fed the high doses. Concomitant with the reduction of the nonacidsoluble enamel matrix in length, a folding of the dentino-enamel junction had occurred. The severity of the folding increased with the increase in dosage of sulfite.

It must be pointed out that there was no correlation between the lack of pigmentation and the histopathologic findings in the enamel organ. In a number of instances (for example, in Group F) the incisors appeared white, whereas the enamel organ appeared normal.

Dentin: In the control animals and those fed up to 0.5 per cent sulfite, formation of dentin was normal. The odontoblasts were of normal height and appearance, and the predentin width was normal on both labial and lingual sides of the tooth.

In two of the rats fed 0.5 per cent sulfite and in all rats that were fed 1.0 per cent sulfite or more, the formation of dentin on the lingual side at the basal one-third of the tooth was severely disturbed leading to a reduction in width (Table I). In most cases the odontoblasts had assumed a spindle shape. No predentin was present and the junction of dentin and pulp was scalloped (Fig. 7). The innermost layer of dentin was stained deeply with hematoxylin.

In all but one of the animals fed 1.0 per cent sulfite or more, some degree of dysfunction of the odontoblasts was seen also on the labial side of the tooth. The pulpal border of the dentin was irregular and covered by irregularly arranged cells.

Odontogenic epithelium: In the control animals and those fed less than 0.5 per cent sulfite, the odontogenic epithelium was entirely normal. In all but 4 of the animals fed higher doses the epithelium invaginated into the pulp cavity toward the lingual formative side (Figs. 7 and 8). In some instances this epithelium reached as far as the middle of the tooth. These epithelial islands and cords induced in some places differentiation of odontoblasts and formation of dentin in the center of the pulp.

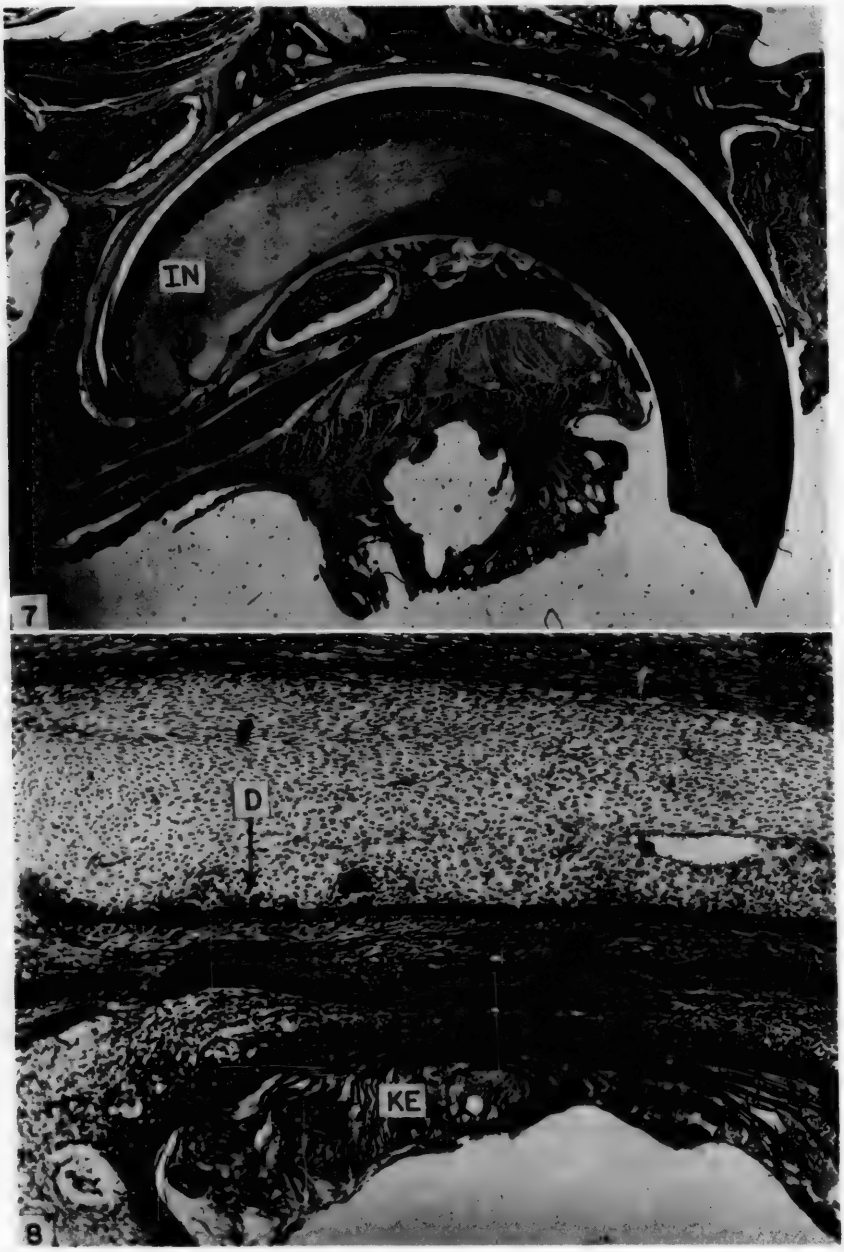


Fig. 7.—Photomicrograph of a central section of an upper incisor of a rat fed 2.0 per cent sulfite for one year. Hematoxylin-eosin stain. Orig. mag. ×10. Note the narrow ligual dentin and the invasion (*IN*) of odontogenic epithelium into the pulp.

Fig. 8.—Higher magnification of the lingual dentin of the basal third of the incisor in Fig. 7. Note the narrow dentin (D), the scalloped border between pulp and dentin and the absence of odontoblasts. The keratinous scales of the metaplastic stratified squamous epithelium (*KE*) of the nasolacrimal duct fills partially the lumen.

The supporting structures of the upper incisor:

1. The Lingual Gingiva. In the controls and in the animals fed less than 0.5 per cent sulfite, the lingual epithelial attachment ended incisally to the alveolar crest. In most of the animals fed higher doses of sulfite, a deep pocket had developed, separating the alveolar crest from the cementum-covered surface of the tooth. In this area the periodontal surface of alveolar bone showed active resorption characterized by Howship's lacunae and large numbers of multinucleated osteoclasts.

2. The Periodontal Membrane at the Lingual Side. In a few instances the periodontal membrane showed reduction in its width and the normal division in 3 layers had disappeared. Principal fibers ran without interruption from alveolar bone to cementum.

3. The Alveolar Bone. The most striking alteration could be seen at the fundus of the alveolar socket in one of the animals of Groups D and H and in all of the animals of Groups K to N. The change consisted of a thickening of the normally extremely thin fundic bone. The apposition of bone began at the periphery and progressed toward the center of the fundic plate. The newly formed bone was separated from the old bone by a distinct resting line and was characterized by a great number of osteocytes and multiple tonguelike spicules which projected toward the tooth. Apposition of bone could also be observed on the labial periodontal surface of the premaxilla, where normally resorption occurs.

4. The Nasolacrimal Duct.* In the control animals and in all but one rat fed less than 0.5 per cent sulfite, the respiratory epithelium of this duct was entirely normal. In all the animals fed higher doses of sulfite, this epithelium was replaced by stratified squamous epithelium, which in some cases underwent keratinization. The changes became more extreme with increased dosage of bisulfite culminating in the plugging of the entire duct by a keratinized mass (Figs. 7 and 8).

DISCUSSION

Animal experiments and chemical investigations reported in the literature pointed to an indirect action of sulfites that had been added to stored foods (Fitzhugh, Knudsen and Nelson[2]). The sulfites did not seem to be toxic in themselves but caused damage by destroying vitamins contained in the diet.

Deficiencies in 3 different vitamins were assumed. Lack of thiamin could be proved by a disappearance of polyneuritis under thiamin management. Vitamin E deficiency was consistent with the findings of "brown uteri." Vitamin A deficiency seemed probable because of lack of pigmentation of the incisors. However, no loss of vitamin A could be detected in the food and no

*In central longitudinal sections through the upper rat incisor an oval cavity is seen within the premaxilla on the concave side of the tooth. This cavity, lined by epithelium, has been variously interpreted as part of the nasal cavity or part of the maxillary sinus. Study of serial sections of the head cut in the coronal plane has shown that this cavity is a cross section of the nasolacrimal duct. This duct starts in the anterior-most region of the orbit, runs forward and slightly downward between the infraorbital nerve and the posterior part of the socket of the upper incisor. It then quite abruptly turns medially, crosses the incisor on its concave surface and opens into the inferior nasal meatus under cover of the maxillo-turbinal bone. The epithelium is pseudostratified ciliated columnar and many small mucous glands are found in the wall of the duct.

sign of vitamin A deficiency could be detected in the many viscera investigated. The typical keratinizing epithelial metaplasias (Wolbach[16]) were looked for in the various organs and were not present except in the lungs, where they sometimes accompanied chronic pneumonia in both treated and control rats.

The present report has shown specific histologic signs of both vitamin E and A deficiency in the rat incisor. The lack of pigment in this tooth and the folding of the dentino-enamel junction, however, are not indicative of any specific deficiency.

Lack of the yellow-brown pigment of the rat incisor has been found under many and various experimental conditions. Deficiency of iron, magnesium and tryptophane, vitamin A, vitamin D and vitamin E; intoxication with cadmium, fluorine, and strontium, and finally after parathyroidectomy.

Folding of the dentino-enamel junction has been observed in all cases where tooth eruption was retarded (hypophysectomy, vitamin A deficiency, excessive incisal stress, etc.). Retardation of tooth eruption is caused primarily by retarded growth of the basal connective tissue of the pulp. The folding of the dentino-enamel junction, therefore, is the result of a disturbance of the normal balance between the rate of growth of the connective tissue and of the enamel epithelium (Sicher[13]).

Signs of Vitamin E Deficiency.—Lack of pigmentation in vitamin E deficiency has been described by Moore[9] and Granados and Dam.[4] According to Irving[8] and Pindborg[10] the premature, sudden and atypical atrophy, the edema, and formation of cysts of the enamel organ are characteristic for vitamin E deficiency. This conclusion was confirmed by the fact that the administration of alpha-tocopherol to rats placed on a diet deficient in vitamin E led to a repair of the described characteristic lesions.

Identical findings in the enamel organs of rats fed higher doses of sodium sulfite permit, therefore, the conclusion that the vitamin E had been eliminated by the addition of the sulfite.

Signs of Vitamin A Deficiency.—It has been shown that vitamin A deficiency causes retardation in eruption (Fredericia and Gudjonsson[3]), metaplasia of different types of epithelium, characteristic changes in the rat incisor (Wolbach and Howe,[14] Schour, Hoffman, and Smith[12]) and in the apposition and resorption pattern of growing bones (Wolbach[15]).

The ciliated epithelium of the nasolacrimal duct of the normal rats had been replaced in the sulfite-fed animals by a highly keratinized stratified squamous epithelium. Desquamated horny scales might almost obliterate the lumen.

Atrophy of the odontoblasts, especially on the lingual side of the incisor, leading to retardation and irregularities of dentin formation, and invasion of the odontogenic epithelium into the pulp, have been shown to be specific for vitamin A deficiency by Wohlbach and Howe, and Schour, Hoffman, and Smith. Replacement therapy with vitamin A corrects these symptoms.

Only a few reports have dealt with the changes in the periodontal membrane and the alveolar bone of the upper incisor in vitamin A deficiency. Schour, Hoffman, and Smith[12] stated that the fundus of the alveolus appeared thicker than normally with prominent spicules of bone, and Irving[7] found a considerable

overproduction of new bone. Apposition occurred according to his findings in some areas where normally resorption takes place as has also been described by Wolbach for other regions of the skeleton. The increased apposition could be seen particularly in the fundic bone, where the new bone showed tongue-shaped outgrowths into the periodontal tissues. A direct comparison of our findings with the illustrations of Irving shows that the changes observed are identical.

Combined Effects of Vitamin A and Vitamin E Deficiency.—The combined occurrence of vitamin A and vitamin E deficiency can be understood from the result of the experiments of Davies and Moore.[1] These investigators showed that vitamin E deficiency led secondarily to vitamin A deficiency. Later it has been demonstrated that vitamin E protects vitamin A and carotene by preventing their oxidation (Quackenbush, Cox, and Steenbock[11]; György and Torravelli[5]; and Hickman, Harris, and Woodside[6]). It seems possible that during storage of the food, vitamin E was destroyed by the sulfite and that consequently vitamin A was oxidized and thus eliminated.

The results of the present experiment are surprising in that they not only confirm the assumption of vitamin E deficiency, but also demonstrate definite signs of vitamin A deficiency. These positive findings are in contrast to those reported earlier (Fitzhugh, Knudsen, and Nelson[2]) in which the evidence of vitamin A deficiency was inconclusive. The explanation for this seeming contradiction is in all probability the high sensitivity of the rat incisor to otherwise subclinical vitamin deficiencies. This susceptibility in turn seems to be founded on the fact that the rat incisor is a continuously forming, growing, and erupting tooth. This entails not only the presence of almost embryologic tissue but also its rapid growth and especially rapid processes of histodifferentiation. This high susceptibility of the rat incisor is consistent with many other observations that fast-growing tissues show a much greater sensitivity to a variety of injuries.

SUMMARY AND CONCLUSIONS

This study is based upon the anatomic and histologic changes of the upper incisors of 43 rats fed a diet containing sulfite ranging from 0.0125 to 2 per cent over a period of from 1 to 2 years.

1. Low doses (up to 0.025 per cent sulfite) produced slight deficiency of pigmentation of the incisor and slight atrophy of the enamel organ.

2. High doses (0.5 to 2.0 per cent sulfite) caused pronounced lack of pigmentation of the enamel; sudden and atypical atrophy of the enamel organ, often accompanied by edema; foldings of the dentino-enamel junction; atrophy and disturbed histodifferentiation of the odontoblasts; retardation and disturbance of dentin formation; invasion of the odontogenic epithelium into the pulp; thickening of the fundic alveolar bone; and keratinization of the epithelium of the nasolacrimal duct.

3. The findings of atypical atrophy and edema of the enamel organ are indicative of vitamin E deficiency.

4. The findings of atrophy of the odontoblasts, invasion of the odontogenic epithelium into the pulp and metaplasia of the epithelium of the nasolacrimal duct are considered to be specific for vitamin A deficiency.

5. The rat incisor is highly sensitive to otherwise subclinical vitamin deficiencies and is therefore useful in assaying different vitamin deficiencies.

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FURTHER STUDIES OF A DIFFERENTIAL CULTURE TECHNIQUE FOR ESTIMATIONS OF ACIDOGENIC BACTERIA IN SALIVA

I. Comparison With Conventional Techniques and Concurrent Clinical Examinations

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THE cultivation of lactobacilli from saliva has been carried out in the past by selective culture methods which suppress the growth of most of the other oral bacteria. Such techniques have great usefulness in estimating the lactobacilli in saliva but have the disadvantage that other bacteria which may conceivably produce enough acid to have significance in relation to enamel decalcification are not counted, for example, the streptococci. Both the tomato agar plate and Snyder's colorimetric test for lactobacilli are adjusted to a hydrogen ion concentration which is generally inhibitory for streptococci. A differential medium for enumeration of lactobacilli was suggested by Rodriguez in 1930,[1] but it never came into use, partly, perhaps, because horse serum is not readily available in sterile form, and partly because anaerobic incubation was used in the procedure. The tomato juice agar of Kulp and White[2] offered the advantages of sterilizable materials and aerobic incubation and was adopted by Hadley,[3] the only change being adjustment to pH 5.0 by means of lactic acid, in order to inhibit more effectively the streptococci. Hadley's method was slightly modified later by the use of glucose infusion broth of pH 5.0 instead of saline as a diluent for the saliva, and by partial neutralization of the mixture of tomato juice, peptone, and peptonized milk before adjustment of the pH to 5.0. Using the procedure thus modified,[4] one seldom finds streptococci on the plates. With a view to making possible the estimation of both the lactobacilli and the streptococci in saliva, Davies, Slack, and Tilden[5] made use of a medium devised by Wade, Smiley, and Boruff.[6] The medium is favorable for both lactobacilli and streptococci, being enriched with ample amounts of glucose, tomato juice, and yeast extract, well buffered, and adjusted to pH 7.0. The only change made in the medium was the substitution of dibasic calcium phosphate for calcium carbonate. The former is especially suitable because it dissolves in the vicinity of the acidogenic colonies only when the pH is reduced to 4.0 By the use of this medium (DST), we have been able to count both lactobacilli and streptococci, differentiating them by the diameter of the clear areas surrounding the colonies. Streptococci rarely produce zones more than 2 to 2.5 mm. in diameter, while lactobacilli, even those of the heterofermentative type, give rise to wide clear haloes (4 to 15 mm.). Separation of colonies is of course necessary in a method of this kind, and we have found it essential to plate two dilutions of saliva (in distilled water), one of 10,000 and one of 100,000.

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It was desirable, of course, to compare the estimates of lactobacilli made by the differential method with those found by the tomato agar plate and with the results of Snyder's colorimetric technique. We were fortunate in securing the cooperation of the supervising personnel of the Chicago Nursery and Half Orphanage Asylum, who permitted us to obtain fasting specimens of saliva whenever we needed them. These were collected under the supervision of the senior author when the children rose at 6 a.m. and were cultured within 3 to 4 hours. The studies were begun in January and February of 1949 with 125 children and included 3 bacteriologic examinations, at intervals of about 2 weeks, and concurrent clinical examinations. The latter were all made by the senior author and were supplemented by bite-wing radiographs. A year later 87 of the same children were available for a second series of examinations, and in January and February, 1951, the 53 children who were still residents of the institution were again studied in the same way as before.

Arnold,[7] in a clinical and bacteriologic survey of 1761 children, found that nearly 50 per cent of children in fluoride-free areas with the highest caries experience fell into the group which had lactobacillus counts of 30,000/ml. or higher, while children whose counts ranged from 100 lactobacilli/ml. to nearly 30,000/ml. occurred with approximately equal frequency in groups with high and low caries experience. On the basis of this observation, we chose 30,000/ml. as the dividing line between high and low or negative counts. If lactobacilli are significant in dental caries because of the acid they produce from carbohydrate residues in the mouth, the lactobacilli must of course reach a certain number in order to produce a significant amount of acid. Beyond this point, however, additional numbers would seem to be of no great significance. The actual numbers of lactobacilli/ml. in our studies were often in the millions. In a child with orthodontic bands, the number may reach 40 to 50 million, as is easily ascertained by suitable dilutions of saliva. One cannot rely on the usual 1:50 dilution of saliva if one is interested in the magnitude of the count. We used 1:50 and 1:250 dilutions for inoculating the tomato agar plates.[8]

Our group was notably different from Arnold's, not only in its size, but also in the fact that whereas Arnold limited his study to children 12 to 14 years old, the ages in our group ranged from 3 to 15 years. Nineteen were in the group of 3 to 6 years, 13 were 12 to 15, and 55 were between 7 and 11. Since the dentition was deciduous and mixed, the criterion used for evaluating the progress of caries was the number of new carious surfaces developed during the study period. Forty-one of the children developed one or more new carious lesions during the first year of study; 46 did not. This division is of course somewhat arbitrary. For example, if a child had lost his deciduous molars because of caries, leaving only the relatively resistant anterior teeth, he might fall into the group showing no new carious lesions. It was essential, however, for statistical purposes, to select some reasonable basis for comparison and adhere to it.*

*Dr. Stanley Harris, of the Physiology Department, Northwestern University Dental School, suggested a treatment of the data which would permit application of the fourfold chi square equation.

Table I shows the result of arranging the data into 4 divisions. The parallelism between the lactobacillus counts and the development of caries was quite striking. Streptococci, on the other hand, were extremely numerous in all the children, both carious and noncarious. There were seldom fewer than 500,000 streptococci/ml., and the more usual count was 5 million or more. Colonies with cleared zones less than 3 mm. were invariably found to be streptococci, as shown in the early part of the study by transferring colonies to blood agar.* Tests on Snyder's medium were made of the streptococcus colonies as well as those of lactobacilli, and it is interesting to note that of the 100 or more streptococci studied, none was found which was able to grow sufficiently on Snyder's medium to change the color of the indicator appreciably. The constant finding of innumerable streptococci in all children recall the data of Hemmens, Blayney, and Harrison[9] with regard to the frequency of streptococci in dental plaques and those of Bibby, Volker, and Van Kesteren,[10] who found that the oral streptococci far outnumbered the oral lactobacilli.

TABLE I

COMPARISON OF CLINICAL AND LABORATORY FINDINGS IN 87 CHILDREN STUDIED FOR A PERIOD OF 12 MONTHS

	BACTERIOLOGY LOW OR NEGATIVE			BACTERIOLOGY HIGH		
	TOMATO AGAR	CALCIUM PHOSPHATE AGAR	SNYDER'S MEDIUM	TOMATO AGAR	CALCIUM PHOSPHATE AGAR	SNYDER'S MEDIUM
No new carious surfaces (46 children)	29	28	23	17	18	23
1 to 8 new carious surfaces (41 children)	12	14	12	29	27	29

	Chi square	*Probability*
For tomato agar count:	9.927	$0.001 < P < 0.01$
For calcium phosphate count:	6.200	$0.01 < P < 0.02$
For Snyder Test:	3.875	$P < 0.05$

About the time of our series of examinations in 1950, certain changes were made at the orphanage with a view to management of the children on a less regimented basis than formerly. A bedtime snack (cookies and milk or crackers and milk) was introduced, and the house mothers no longer supervised so strictly the bedtime toothbrushing. How much these measures influenced the progress of caries is impossible to evaluate. If current theories of dental caries are correct, however, they were an important factor, and the results of our third series of clinical and bacteriologic examinations bear out this assumption. Clinical examinations were recorded of the 53 children remaining from the previous year's 87. On the basis of the occurrence of new carious lesions, only 4 children had failed to develop new lesions. Of the 49 children who showed progress of caries, 45 had high lactobacillus counts.

Although no statistical evaluation is possible with data such as these, it is interesting to consider the data for the entire period of 2 years, as shown in

*Blood agar containing 5 per cent sucrose was used, because this medium is a convenient one for recognizing *Streptococcus salivarius*. The great majority of the salivary streptococci proved to be of this species.

Table II. If parallelism between clinical findings and bacteriologic examinations be taken as the test of the significance of the latter, then the acidogenic tests would definitely seem to be superior to the tomato agar count, since they detect conditions presumably predisposing to caries in a larger percentage of cases and considerably earlier.

TABLE II

DATA ON 53 CHILDREN STUDIED FOR 24 MONTHS
3 SAMPLES OF SALIVA FROM EACH ANNUALLY AT INTERVALS OF 2 WEEKS

1949	37 had high counts
	19 by acidogenic tests only, 18 also on tomato agar
1950	38 had high counts
	10 by acidogenic tests only, 28 also on tomato agar
	17 with high counts had no new carious lesions
	5 with new carious lesions had negative counts
1951	45 had high counts
	7 by acidogenic tests only, tomato agar negative, all 7 had new carious lesions
	8 had negative counts, 5 of whom had new carious lesions
	3 of the latter 5 have had negative counts throughout the 2 year period

As noted in Table II, the saliva of an occasional child with developing carious lesions showed no lactobacilli by any method of cultivation. The same puzzling situation was seen in the group of teen-aged girls whom we studied in 1950 and 1951 (Table III). In this group the Snyder test was the only indication of the presence of acidogenic bacteria in 3 of the 6 girls who had new carious lesions, since no lactobacilli were found in these 6 girls by either of the quantitative methods. For this reason, the statistical significance of the results of the 3 culture methods is reversed (compare Table I).

TABLE III

COMPARISON OF CLINICAL AND LABORATORY FINDINGS IN 25 TEEN-AGED GIRLS STUDIED FOR 12 MONTHS

	BACTERIOLOGY LOW OR NEGATIVE			BACTERIOLOGY HIGH		
	TOMATO AGAR	CALCIUM PHOSPHATE AGAR	SNYDER'S MEDIUM	TOMATO AGAR	CALCIUM PHOSPHATE AGAR	SNYDER'S MEDIUM
No new carious surfaces (10)	9	9	9	1	1	1
1 to 10 new carious surfaces (15)	6	6	3	9	9	12

	Chi square	*Probability*
For tomato agar count:	6.25	$0.01 < P < 0.02$
For calcium phosphate count:	6.25	$0.01 < P < 0.02$
For Snyder test:	9.85	$0.001 < P < 0.01$

Recently Rogosa, Mitchell, and Wiseman[11] have worked out a very highly selective medium for lactobacilli which satisfies the nutrient requirements for all lactobacilli. It is not at all differential; all lactobacilli grow on it very abundantly. It is unfavorable for the growth of sporeformers and for molds and yeasts. It does not need to be sterilized but can be dispensed hot in flasks. We have kept it for weeks in the refrigerator. Dr. Rogosa, in furnishing the

instructions for preparation of the medium ("SL" medium) in advance of publication, permitted us to compare this method with the other 3 in our series of examinations in 1951. Agreement was virtually complete in all instances, including the 3 children mentioned in Table II and the 3 teen-aged girls in Table III, who had progressive caries but no lactobacilli. The counts were somewhat higher on the "SL" medium than on the others, presumably because the medium is more specifically adapted to lactobacilli.

DISCUSSION

Employment of several methods of culturing lactobacilli from saliva has emphasized again the notable coincidence between the presence of large numbers of lactobacilli in the saliva and the progress of dental caries. Estimation of streptococci failed to reveal any such parallelism between carious conditions and the presence of streptococci, which appeared to reside in the mouths of all the children and to proliferate in enormous numbers. Stephan and Hemmens[12] found in experiments with cultures of oral lactobacilli and other oral bacteria, such as the staphylococci and streptococci, that the lactobacilli, when large numbers of cells were exposed to molar concentrations of glucose, were capable of producing and maintaining a high concentration of hydrogen ions, while cocci, under similar conditions, gave rise to considerable acid, which disappeared after a short time. Stephan and Hemmens suggested that the disappearance of the acid formed might be due to its utilization by the cocci. De Jong,[13] however, found that *Micrococcus albus* was unable to metabolize organic acids, hence it would be interesting to investigate the ability of oral streptococci to utilize lactic and other organic acids.

The absence of lactobacilli from the saliva of 3 children who showed progressive caries over a period of 2 years has not been explained. In view of the rather exhaustive bacteriologic study, which included at least 3 tests each year by 3 different procedures (including, as fourth test, that of Rogosa, Mitchell and Wiseman[11] the third year), it does not seem likely that the lactobacilli were missed if they were present in the saliva. Cultural studies of bacterial plaques might have revealed that the lactobacilli were present in large numbers in these important areas. The plaques are difficult to remove from the teeth and occur most often in areas not reached by saliva. Until a study has been made of such cases as these with both saliva and plaque material, it can hardly be concluded that the results minimize the role of the lactobacilli in dental caries.

SUMMARY

A group of orphanage children, the majority 3 to 11 years old, were followed by annual clinical and bacteriologic examinations; 87 children for one year and 53 for two years. The figures for the standard tomato agar count, the Snyder test, and the acidogenic count introduced by Davies, Slack, and Tilden (DST medium) were compared statistically with the progress of caries, as indicated by the number of new carious lesions revealed by clinical and radiographic examination. The findings corroborated once more the striking associa-

tion of carious conditions with the presence of large numbers of lactobacilli in the saliva. A similar striking parallelism was observed in a one year study of a group of 25 teen-age girls.

Streptococcus counts on DST medium were very high in all the children studied, irrespective of clinical conditions; there was no indication in these experiments that the streptococci are significant in relation to dental caries.

The DST count and the Snyder test were positive in some instances much earlier than the tomato agar count, perhaps indicating a slightly greater efficiency of the acidogenic tests in anticipating the onset of caries.

A few puzzling cases were found in which caries developed in the apparent absence of lactobacilli. It is suggested that counts of lactobacilli in bacterial plaques should be made in such cases as these.

ADDENDUM

We were able to secure tooth scrapings, in June, 1952, from 1 of the 3 children who had continued to develop new carious lesions throughout the 2 years of study but had shown no lactobacilli in the saliva at any time. The scrapings were added to 5 ml. of acid broth, and 1:10, 1:100, and 1:1,000 dilutions of this mixture were plated on tomato agar. Abundant growth of lactobacilli occurred on the plate inoculated with the highest dilution. At this time the child had 3 carious lesions not present at the examination made in 1951.

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FURTHER STUDIES OF A DIFFERENTIAL CULTURE TECHNIQUE FOR ESTIMATIONS OF ACIDOGENIC BACTERIA IN SALIVA

II. Species of Lactobacilli Isolated From Saliva and Their Distribution In A Group of Children

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THE taxonomic characters of the lactobacilli, as set forth in Bergey's Manual,[1] are presumably based on studies of the species isolated from such natural sources as fermented milks, cheese, sauerkraut, pickles, and the human intestine. These lactobacilli are of 2 main groups. The homofermentative varieties, so called because they produce lactic acid almost exclusively when acting on glucose, are distinguished from one another by the kind of lactic acid formed (dextro, levo, or inactive), by their optimal temperatures for growth, and finally, by the kinds of carbohydrates which they ferment. The second group of lactobacilli, those which produce carbon dioxide and other acids as well as lactic, are referred to as the heterofermentative species and are distinguished from one another by temperature requirements and by fermentation reactions with various carbohydrates. Until recently,[2, 3] the oral lactobacilli have not been carefully studied from the taxonomic viewpoint.

IDENTIFICATION OF LACTOBACILLI ISOLATED FROM SALIVAS OF CHILDREN

In connection with the experiments described in the first part of this report,[4] it became desirable to identify bacteria producing zones of clearing on the DST plates containing calcium phosphate.[5] The identification was made by determining the following properties: (1) maximum production of acid on infusion broth containing an excess of glucose (2 per cent); (2) capacity to produce acid, or acid and gas, on Snyder's medium (glucose agar of pH 5.0 with brom cresol green as indicator); (3) action on milk; and (4) action on various carbohydrates. The techniques used were those employed by Tittsler, Geib, and Rogosa,[6] in their studies of lactobacilli.* All cultures were tested for catalase activity, since lactobacilli are known to be catalase negative. Tests for the type of lactic acid produced were not made. This had been done in the study made by Rogosa, Mitchell and Fitzgerald,[2] and had been correlated with fermentation tests, hence we were able to rely on the latter.

In the early work the isolations of lactobacilli were all made from acidogenic colonies growing on the DST medium. To our surprise, when some of the pure cultures of lactobacilli thus obtained were tested on Snyder's medium, they produced both acid and gas, and when fermentation tests were made, it was found that most of these gas-forming lactobacilli were *L. fermenti*. At the time

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*The paper by these authors has been published only in abstract, and we are indebted to Dr. Tittsler for sending us details of the methods used.

of our second series of examinations, the Snyder tests were examined carefully for bubbles of gas, and it was found that gas-forming lactobacilli were present in more than 50 per cent of the children. Finally, at the third annual examination, the technique was broadened to make possible a survey of the distribution of the heterofermentative lactobacilli as compared with the homofermentative types. Isolations were made not only from acidogenic colonies on the DST plates, but representative colonies were picked from tomato agar, and whenever the Snyder test showed gas, a subculture was made from the Snyder tube directly to infusion broth containing an excess of the two carbohydrates commonly fermented by the gas-producing lactobacilli, namely, raffinose and xylose. These broth cultures were then plated on tomato agar. This last technique resulted in our obtaining a larger number of the heterofermentative lactobacilli (Table I), though we were able often to pick them also from the original tomato agar plates because of the peculiar "ground-glass" appearance which their colonies usually showed on this medium (Figs. 7 and 10). The acidophilus, it will be noted, was obtained only from tomato agar*; it is not sufficiently acidogenic to produce zones of clearing on the DST medium or to change appreciably the color of the indicator in Snyder's medium.

TABLE I

RESULTS OF CULTURING LACTOBACILLI FROM SALIVA BY VARIOUS PROCEDURES

	ACIDOPHILUS	BUCHNERI	BREVIS	FERMENTI	PLANTARUM	CASEI	LACTOSE-NEG.
Calcium phosphate plate (DST medium)	0	0	0	11	4	37	4
Tomato agar plate	4	6	6	28	11	64	12
Snyder's medium via raffinose or xylose broth and tomato agar	0	3	0	15	1	23	3

DISTRIBUTION OF SPECIES OF LACTOBACILLI IN CHILDREN

The strongly acidogenic lactobacillus, *L. casei,* proved to be the predominant species, being present (Table II) either alone or with other species in 75 per cent of the 53 children (88 per cent of the 45 who had high lactobacillus counts). *L. plantarum,* another strongly acidogenic homofermentative species, was sometimes present, but much less commonly. The occasional lactose-negative cultures isolated are similar in fermentation reactions, in final pH attained in glucose broth, and in nutrient requirements, to the rhamnose-negative cultures of *L. casei.*† However, they were often recovered in several successive cultures from the same child, sometimes in 2 or more annual examinations. If they are variants of *L. casei,* they were remarkably constant in their reactions. Whatever the original source of the lactobacilli in the saliva (presumably the food), there is indication that one or another species is capable of eventually

*Addition of 20 mg. of actidione (Upjohn) to 100 ml. of tomato agar has no effect on the growth of lactobacilli but inhibits such common molds as *Aspergillus niger* and *Penicillium notatum.* We used it routinely in our last series of cultures (1951) and have used it also in our class work on lactobacillus counting. It does not appear to inhibit *Candida albicans.*

†The majority of the cultures of *L. casei* (approximately 70 per cent) fermented rhamnose.

establishing itself in the mouth and remaining there for some time. Sometimes a variety of species is obtained from the same child, 3 in several instances (Table III).

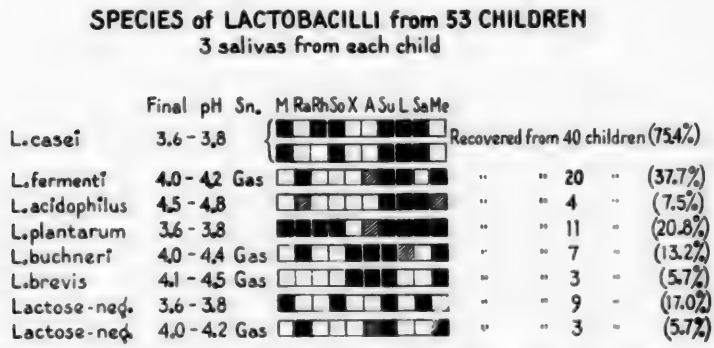
SPECIES of LACTOBACILLI from 53 CHILDREN
3 salivas from each child
Final pH Sn. M Ra Rh So X A Su L Sa Me
L. casei 3.6 - 3.8
Recovered from 40 children (75.4%)
L. fermenti 4.0 - 4.2 Gas " " 20 " (37.7%)
L. acidophilus 4.5 - 4.8 " " 4 " (7.5%)
L. plantarum 3.6 - 3.8 " " 11 " (20.8%)
L. buchneri 4.0 - 4.4 Gas " " 7 " (13.2%)
L. brevis 4.1 - 4.5 Gas " " 3 " (5.7%)
Lactose-neg. 3.6 - 3.8 " " 9 " (17.0%)
Lactose-neg. 4.0 - 4.2 Gas " " 3 " (5.7%)

Table II.—Characteristics of various species of lactobacilli isolated and their incidence in the 53 children studied in 1951 (8 of these children were negative for lactobacilli). Black and white blocks are used to indicate fermentation and no fermentation, respectively. Cross hatching indicates that some strains fermented this carbohydrate, while others did not. The carbohydrates, in the order given, are: Mannitol, raffinose, rhamnose, sorbitol, xylose, arabinose, sucrose, lactose, salicin, and melibiose. Sn = Snyder's medium.

TABLE III

DISTRIBUTION OF THE VARIOUS SPECIES OF LACTOBACILLI IN THE CHILDREN WHO SHOWED HIGH LACTOBACILLUS COUNTS IN 1951

Casei alone	12 children
Casei and plantarum	5 children
Casei and fermenti	9 children
Casei and buchneri	2 children
Casei fermenti and buchneri	2 children
Casei fermenti and acidophilus	3 children
Casei fermenti and plantarum	4 children
Casei fermenti and brevis	1 child
Casei fermenti, buchneri, brevis	1 child
Casei buchneri and acidophilus	1 child
Plantarum and brevis	1 child
Plantarum alone	1 child
Buchneri alone	1 child
Lactose negative alone	2 children

NUTRIENT REQUIREMENTS OF HOMOFERMENTATIVE AND HETEROFERMENTATIVE LACTOBACILLI

The nutritive requirements of many of the lactobacilli have been worked out in detail in connection with their employment in microbiological assays. Some of the earlier synthetic media devised for this purpose were designed to meet the nutrient requirements of *L. casei* and do not support the growth of many other lactobacilli. That of Shankman, Dunn, and Rubin[7] for example, would permit growth and production of acid by *L. casei* 7469 and *L. plantarum* 8014 but not by the following species of lactobacilli obtained from the American Type Culture Collection: *L. delbrueckii* 9649, *L. leichmannii** 4797, *L. fermenti*

*This culture was lactose-positive in our hands.

9338, *L. brevis* 8257, *L. buchneri* 4005, and 11 strains of *L. acidophilus.** The oral lactobacilli which we had indentified as *L. casei* or *L. plantarum* also grew on this medium and gave a titration similar to that given by the ATCC cultures of these organisms.† Our lactose-negative cultures behaved like *L. casei* on the medium of Shankman and others, another indication perhaps that they are variants of this species. The heterofermentative lactobacilli isolated from the

Titration of Lactic Acid Produced on Assay Medium (Shankman et al)

No. of cultures		Final pH	Sn. M Ra Rh So X A Su L Sa Me	Acid production ml. 0.02 N NaOH
89	L. casei	3.6 - 3.8		4 - 5
23	L. plantarum	3.6 - 3.8		4 - 5
12	Lactose-neg.	3.8 - 3.9		4 - 5
46	L. fermenti	4.0 - 4.2 Gas		none
12	L. buchneri	4.0 - 4.4 Gas		"
4	L. brevis	4.0 - 5.8 Gas		"
3	L. acidophilus	4.6 - 5.1		"
11	" (ATCC)	4.8 - 5.2		"

Table IV.—Correlation of properties of lactobacilli isolated from saliva with their nutritive requirements as shown by acid production on a medium devised for microbiological assay by means of *L. casei*.[7] For explanation of abbreviations see legend for Table II.

Titration of Acid Produced on Assay Medium (Dunn et al)

No. of cultures		Final pH	Sn M Ra Rh So X A Su L Sa Me	Acid production ml. 0.02 N NaOH
40	L. fermenti	4.0 - 4.2 Gas		14 - 29
4	L. buchneri	4.1 - 4.2 "		16 - 18
4	" "	4.1 - 4.6 "		0.5 - 1.5
3	L. brevis	4.1 - 4.4 "		16 - 24
4	" "	4.0 - 4.5 "		4 - 7.6
	L. casei 7469	3.7		15.0
	L. fermenti 9338	4.15 Gas		16.5
	L. buchneri 4005	4.35 "		21.2
	L. brevis 8257	5.95 "		10.7

Table V.—Correlation of the properties of the gas-producing lactobacilli isolated from saliva with their nutritive requirements as shown by acid production on a medium devised for microbiological assay by means of *L. fermenti*.[8] For explanation of abbreviations, see legend for Table II.

*The ATCC numbers of these cultures were as follows: 314, 332, 832, 4356, 4796, 4913, 4962, 4963, 4355, 4357, and 9857.

†We included in the group of cultures tested 6 cultures of *L. casei* obtained from root canals (Tulacek, R. S., and Tilden, E. B., *J. D. Res.* **26**: 247, 1947). These cultures behaved in every way like the cultures of *L. casei* obtained from the salivas of the children.

children failed to grow on this medium, as did also our strains of *L. acidophilus;* blank titrations were obtained with all of these cultures (Table IV).

On the other hand, the medium devised by Dunn, Shankman and Camien[8] for *L. fermenti* supported growth of most of the heterofermentative lactobacilli which we isolated, and considerable amounts of acid were produced (Table V). The titrations showed a wider range than those obtained on the medium which supported only the homofermentative types. This medium, like most of the more recently devised synthetic media for microbiologic assay, supports the growth of a much larger number of species; *L. casei* grows well on it and gives a higher titration than on the medium of Shankman, Dunn and Rubin.

REACTIONS OF PURE CULTURES OF LACTOBACILLI ON SNYDER'S MEDIUM

If one uses the Snyder medium as we did, in testing pure cultures for gas production, it is found that even the strongly acidogenic species may not always produce a four plus reaction in 24 hours, or even in 48 hours, and the heterofermentative species, as would be expected, often do not produce a complete change of the indicator even in 72 hours (Table VI). These findings may have some significance for the clinical use of the medium. In our clinical study,[4] we read the Snyder tests according to the usual practice, that is, we disregarded 3+ reactions altogether. The gas-producing lactobacilli are sufficiently acidogenic to produce wide zones of clearing on DST medium. Moreover, they remain viable for at least 10 days on 2 per cent glucose broth (as do all lactobacilli, unlike the streptococci). Hence their acidogenic and aciduric capacities are not greatly inferior to those of the homofermentative species. It is suggested, therefore, that in the clinical use of the Snyder test, if gas bubbles are found to be present,* a 3+ reaction may be just as significant as a 4+, either at 48 or at 72 hours.

TABLE VI

REACTIONS OF PURE CULTURES OF LACTOBACILLI ON SNYDER'S MEDIUM

	4 PLUS REACTION			3 PLUS REACTION	LESS THAN 3 PLUS
	24 HRS.	48 HRS.	72 HRS.	72 HRS.	72 HRS.
Casei	37	25	21	23	13
Plantarum	10	4	4	2	4
Lactose-neg.	5	1	1	1	1
Fermenti	1	5	20	15	1
Buchneri and brevis			3	5	
Acidophilus					4

RECOGNITION OF VARIOUS SPECIES OF LACTOBACILLI ON TOMATO AGAR

The colonial morphology of the oral lactobacilli, described by earlier workers as variants of *L. acidophilus,* was found by us to be characteristic of certain species, to the extent that we were often able to differentiate the homofermentative from the heterofermentative species by the appearance of their colonies on tomato agar. The heterofermentative species are usually, though not always, recognized by their irregularity of margin and "ground glass" texture (Figs.

*The growth of yeasts on Snyder's medium is readily distinguished from growth of lactobacilli.

7, 8, 9 and 10). By contrast, the smooth colonies of *L. casei* and *L. plantarum* have a regular margin and a homogeneous opaque appearance (Figs. 1 and 2), though small colonies of *casei* may be almost transparent (Fig. 3). Colonies of *L. plantarum* (Fig. 2) show a definite tendency to be larger than those of *L. casei*. *L. casei,* as well as the lactose negative lactobacilli which we isolated, are very prone to assume a kind of "fried egg" colony (Fig. 4), which may simply be a transitional type between the typical smooth (Fig. 1) and the rough (Fig. 5). Rough colonies are much more difficult to differentiate than smooth ones, though a ground glass appearance is sometimes detectable in rough colonies of heterofermentative species (Fig. 10). Colonies of *L. fermenti,* which grows more rapidly than *L. casei,* tend to be rather large; Fig. 6 shows a smooth and a rough colony of *L. casei* and a typical ground glass colony of *L. fermenti,* for comparison. Some very large colonies of *L. fermenti* are shown in Figs. 7, 8, and 9. This species tends to show a marked yellowish pigmentation in old cultures. *L. buchneri* varies very much in colony size (Figs. 10 and 11), as does *L. brevis,* (Figs. 12 and 13), and the ground glass appearance is perhaps less striking in these species than in *L. fermenti.* The few strains of *L. acidophilus,* we have isolated, have always shown exclusively the rough colony (Fig. 14) which, according to Tittsler, Geib, and Rogosa[6] is characteristic of this species.

DISCUSSION

Our studies of the oral lactobacilli reveal that the variations in fermentation reactions and acidogenic capacity reported by Hadley, Bunting, and Delves,[9] by Sullivan, Still and Goldsworthy,[10] and by Clapper and Heatherman[11] are in reality characteristic of individual species of lactobacilli, homofermentative and heterofermentative, which are present in the oral cavity. Separation of the species is not an easy matter, and often many platings and replatings are necessary before the fermentation reactions become stabilized. The finding of one or two characteristic colonies of heterofermentative types on a plate otherwise characteristic of *L. casei* is often found to be the explanation of a positive reaction on raffinose or melibiose, which are not fermented by *L. casei.* A very slight contamination of a homofermentative by a heterofermentative culture may not be revealed in the test for gas production, and the separation of *L. plantarum* from the heterofermentative species is especially difficult, because a mixture of *L. casei* and *L. fermenti* gives fermentation reactions identical with those of *L. plantarum.* The morphology of colonies on tomato agar is often helpful in purification of mixed cultures.

The distribution of the various species in the individual children was probably not characteristic particularly of the salivas of children; a similar distribution was found in a group of teen-aged girls who were also studied, and the percentages of the various species are quite similar in our children to those reported by Rogosa, Mitchell, and Fitzgerald[2] in a study of many saliva samples.

SUMMARY

The predominant species of lactobacillus in the salivas of children was the strongly acidogenic *L. casei,* which was present alone or with other species in 88 per cent of the 45 children with high lactobacillus counts.

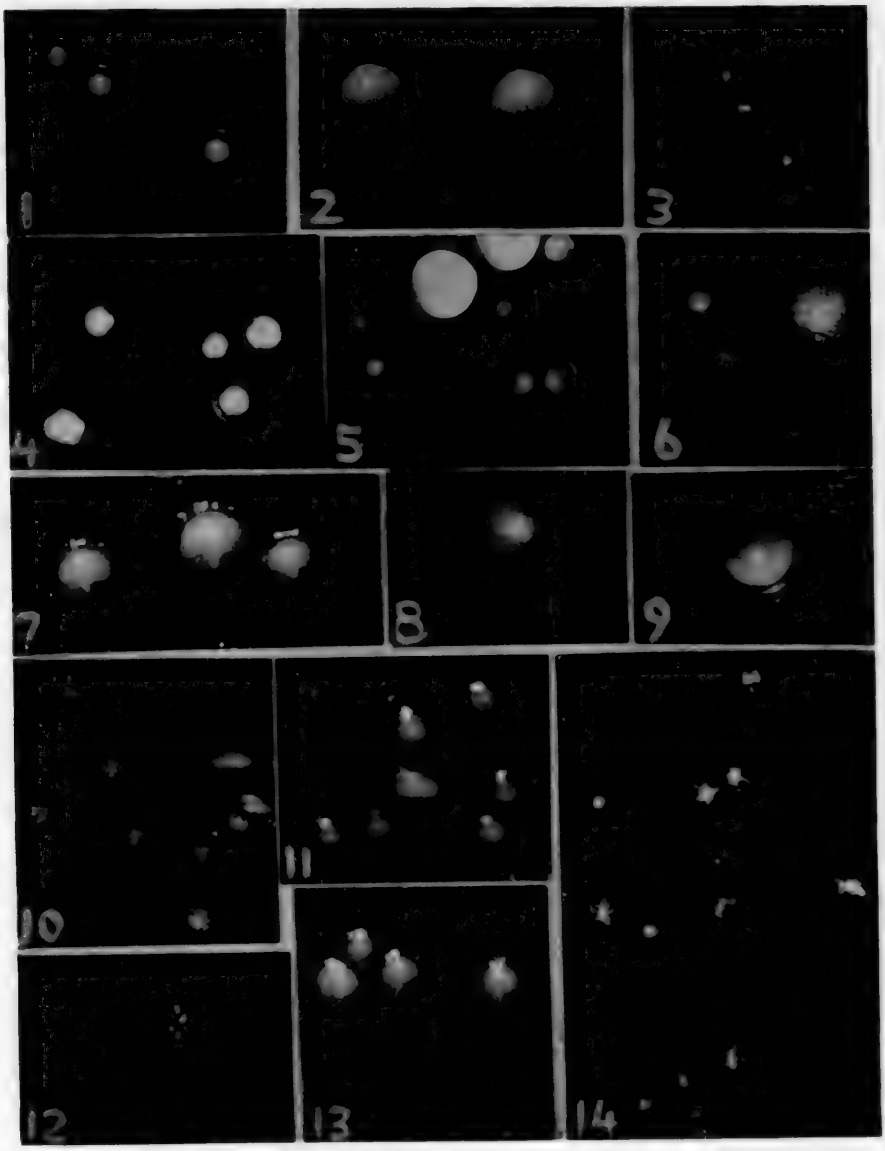

Fig. 1.—Typical smooth colonies of *L. casei*.
Fig. 2.—Typical smooth colonies of *L. plantarum*.
Fig. 3.—*L. casei*, small type of smooth colony.
Fig. 4.—*L. casei*, "fried-egg" colonies.
Fig. 5.—*L. casei*, "rough" colonies.
Fig. 6.—A rough and a smooth colony of *L. casei* and a typical "ground-glass" colony of *L. fermenti*, from a mixed culture.
Figs. 7 and 8.—Typical "ground-glass" colonies of *L. fermenti*.
Fig. 9.—*L. fermenti*, less usual type.
Fig. 10.—Rough colonies of *L. buchneri*, showing "ground-glass" appearance.
Fig. 11.—Smooth colonies of *L. buchneri*.
Fig. 12.—Rough colony of *L. brevis*.
Fig. 13.—Smooth colonies of *L. brevis*.
Fig. 14.—Typical rough colonies of *L. acidophilus*.

Gas-producing lactobacilli were present in more than half of the children, occasionally alone, but more commonly with other species. The production of gas in the Snyder test is an indication of their presence. *L. fermenti* was the most frequently encountered of the heterofermentative lactobacilli, being present in more than 40 per cent of the 45 children with high lactobacillus counts. Gas-producing lactobacilli in pure culture often do not produce a 4+ reading on Snyder's medium, even in 72 hours.

Lactobacillus acidophilus was isolated from only a small percentage of the children and only from tomato agar plates. This species is not sufficiently acidogenic to produce any appreciable change of color in Snyder's test or any cleared zones on DST medium.

A number of lactose-negative lactobacilli were isolated which were distinct from the two lactose-negative species already known (*L. leichmannii* and *L. delbrueckii*). They resembled *L. casei* in all respects except in the failure to ferment lactose.

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DENTAL CARIES EXPERIENCE IN PRIMARY TEETH

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COMPARATIVELY little information is available about dental caries experience in primary teeth, particularly in children before the age of 5 and after the age of 9. In this regard it seems useful to describe two sets of data that have come to hand. With due allowances for differences in attack rates and amounts of care, this material can be combined to present an instructive picture of caries attack in primary teeth through a full cycle from eruption to exfoliation. In both sets of material, all of the examinations were made by full-time staff dentists trained in standard methods of recording all important conditions in the 20 primary tooth spaces.

During the period, 1939 to 1942, the Division of Dental Health of the Illinois Department of Public Health examined the mouths of some 3,000 children from 1 month to 71 months of age. These children were examined when their parents brought them to Well Baby Conferences held at the annual state fairs. The children were from many parts of the state, though the extent to which they may have represented Illinois children in general is not known. It is possible that the children were more representative of rural than urban groups, but this is uncertain. Since attendance at the conferences was voluntary, one may believe that pride and concern of the parents regarding their children's health were, to some extent, factors affecting participation in the examinations. So far as oral conditions were concerned, it seems doubtful that the circumstances of the conferences would markedly bias the sample.

The Departmental dentists were making these examinations in order to verify the eruption times of teeth, but they simultaneously recorded the other important conditions of the teeth. Dr. John E. Crietzberg, Director of the Division of Dental Health in Illinois, has kindly given me permission to analyze the summary sheets of the findings. They afford good information, not only regarding eruption, but regarding decayed, missing, and filled primary teeth as well. Since no significant number of primary teeth are exfoliated normally up to age 54 months, any teeth missing by that age were counted as lost because of decay or injury. Between 54 and 60 months a few teeth had been lost by normal exfoliation, but they were practically always distinguishable from those lost through disease. Thus, up to 60 months of age, the "cross-sectional" data available for the Illinois children were suitable for the statistical needs of this report.

The Cleveland Board of Education, which has conducted an extensive dental program for many years, permitted me to examine more than 3,000 dental records of school children in that city. This material is particularly useful because of

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its serial or longitudinal character. Each record contains information on the periodic dental examinations, given at intervals of, roughly, one year. With such material it is possible to study the same tooth space of the same child for a number of years.

Particularly with records of children who had examinations before reaching 60 months of age, valid judgments could be made as to whether any given primary tooth had been lost through disease or through the normal processes of exfoliation. Beginning at about 60 months, this problem becomes important, and cannot be dealt with satisfactorily except with longitudinal data like that available on the Cleveland children. The records of 233 of the Cleveland school children were therefore analyzed throughout the age range 60 to 156 months. The representativeness of these children, too, is uncertain, but the data are not believed subject to marked bias because the only important factor in their selection was whether the children had been examined early enough and often enough to permit detailed analysis of the records.

In organizing the data from both areas, the various ages in months were grouped around mid-points at 6, 18, 30, 42, etc. All ages at time of examination below 12 months were grouped at 6 months, all ages from 12 to 23 months, inclusive, were grouped at 18 months, all ages from 24 to 35 months, inclusive, were grouped at 30 months, etc.

The examination findings in each of the 20 tooth spaces for all children in each of these age groups were totaled for sound (s), decayed (d), missing and indicated for extraction (e), and filled (f). A count of the number of erupted teeth, at each age, was made for the Illinois children.

As already indicated, for the Cleveland children it was feasible and necessary to distinguish between (a) "essential" teeth, which were the only ones scored as *s, d, e,* or *f;* and (b) "exfoliated" or unessential teeth. Teeth counted in class (b) included not only teeth actually exfoliated within a normal time before eruption of their permanent successors, but also any primary teeth (regardless of their condition) that remained in the mouth longer than the time assumed to be normal for exfoliation.

There is no complete agreement as to the normal interval between exfoliation and eruption. It was convenient in our data to assume 6 months for that interval, in view of the fact that the examinations were made approximately once a year. However, the meaning of our statistical results can be judged fairly well for any other assumed interval, such as 8 months, and if necessary some statistical adjustment might be made for that assumption. The effect would be a small lowering of the upper line shown in the right-hand part of the chart; otherwise the basic picture would be changed very little.

The actual eruption of a permanent tooth might have occurred at any time between the examination when it was first recorded and the preceding examination. Since the preceding examination was usually one year earlier, the best estimate of the actual (average) eruption time was 6 months before the examination in which eruption was first noted.

In deciding whether a given primary tooth space, during the period before eruption, should be considered essential or exfoliated as defined above, account

was taken of the data available for the year preceding the first recorded eruption. For each tooth space up to and including the 12 months preceding the first recorded eruption, the 6-month-average rule required that the tooth be counted as exfoliated; for a period of 13 months or more before the first recorded eruption the tooth was counted as essential and scored as *s*, *d*, *e*, or *f*. Admittedly, this rule would often be quite inaccurate where interest centered on a particular tooth in a particular child's mouth. For statistical purposes, however, it is apparent that the errors tend to cancel out rapidly with hundreds of cases, and make for a reliable estimate of the typical picture.

Using the data assembled in this manner, rates were calculated for the conditions found in each group of children. The results are given in the table and presented graphically in the chart. Separate rates were studied for male and female but, since the sex differences were small and in the usual direction, the data are combined in this presentation. All children in this study were white.

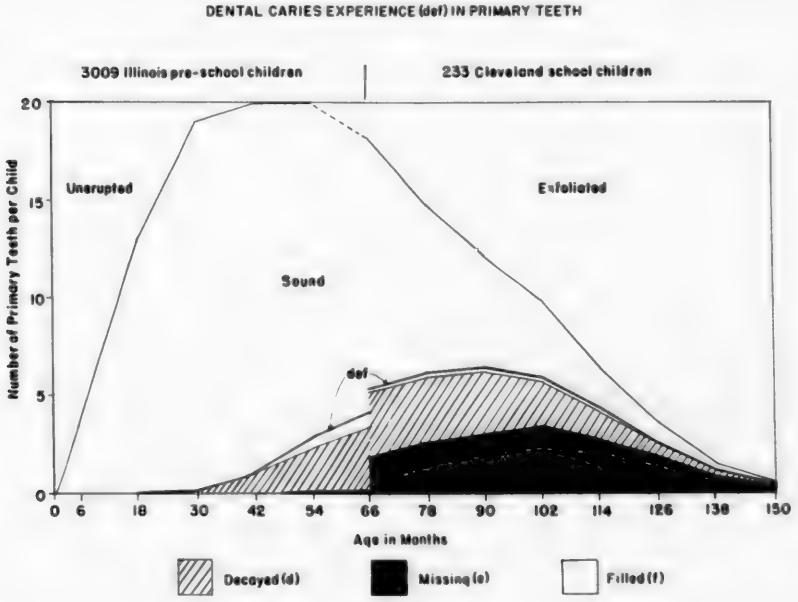

Fig. 1.

The shape of the upper curve shown in Fig. 1 emphasizes the rapidly changing nature of the population of primary teeth in the mouth. Although this curve covers an age range of 13 years, at only ages 42 to 54 months are all 20 teeth present. The chart as a whole shows the desirability of expressing, wherever possible, the caries experience for primary teeth as rates per 100 essential, teeth, as well as per child, in order to be fully descriptive. The next to last column of Table I illustrates the *def* rate per child, and indicates a slowing down of caries activity at 66 months of age, a peak at 90 months, and a rapid

TABLE I

DENTAL CARIES IN PRIMARY TEETH 3,009 ILLINOIS PRESCHOOL AND 233 CLEVELAND SCHOOL CHILDREN

AGE IN MONTHS	NUMBER							TEETH PER CHILD			DEF PER 100 ESSENTIAL TEETH
	CHILDREN	TEETH									
		ESSENTIAL	SOUND	d	e	f	def	ESSENTIAL	SOUND	def	
						Illinois					
6	163	560	560	0	0	0	0	3.44	3.44	0.00	0.0
18	313	3,998	3,991	7	0	0	7	12.77	12.75	0.02	0.1
30	285	5,366	5,302	52	0	12	64	18.83	18.60	0.22	1.1
42	277	5,516	5,222	260	8	26	294	19.91	18.85	1.06	5.3
54	433	8,631	7,411	1,019	32	169	1,220	19.93	17.12	2.82	14.1
66	1,538	29,738	23,463	4,859	363	1,053	6,275	19.34	15.26	4.07	21.1
						Cleveland					
66	233	4,214	2,960	798	427	29	1,254	18.09	12.70	5.38	29.7
78	233	3,438	1,995	778	613	52	1,443	14.76	8.56	6.19	41.9
90	233	2,869	1,371	714	729	55	1,498	12.31	5.88	6.43	52.2
102	233	2,331	966	535	776	54	1,365	10.00	4.15	5.86	58.5
114	233	1,549	530	345	632	42	1,019	6.65	2.27	4.37	65.7
126	233	880	263	181	416	20	617	3.78	1.13	2.65	70.1
138	233	380	85	76	208	11	295	1.63	0.36	1.27	77.6
150	233	150	26	26	93	5	124	0.64	0.11	0.53	82.6

misprint

decline thereafter. When *def* is considered in terms of essential teeth, however, it is clear that attack rates are rising as long as teeth are present.

The difference in the attack rates for the Illinois and Cleveland children appears at 166 months, the age where the two groups overlap. The average Cleveland child at this age has 1.3 more *def* teeth than his Illinois counterpart. The *def* rate per 100 essential teeth is also 8.6 higher in Cleveland.

The general nature of the curves for dental caries experience is consistent for the two groups, however. If it were possible to extend these curves for either group of children they would probably indicate findings of the kind observed in comparing various DMF curves; although the attack rates may differ at any given age, if there is no sample bias the DMF curves tend to parallel each other. The present data suggest that dental caries attack in primary teeth follows the pattern found in permanent teeth, in that attack lags behind eruption but rises as posteruptive tooth age increases. The longer teeth remain in the mouth, the more likely they are to show evidence of caries attack.

It will be noted that the black area shown in Fig. 1 for the Cleveland children has been subdivided by a white dotted line. Only the black area below the dotted line is comparable with the black band shown for the Illinois children. The black area above the dotted line represents those teeth which, although still present in the mouth, were counted by the Cleveland examiners as beyond repair and indicated for extraction. Since that group of teeth was not distinguished from other decayed teeth in the Illinois children, no comparable band can be shown in the left-hand part of the chart. Although it can be assumed that a significant band representing teeth of that kind existed for the Illinois children, it is hardly likely that its width at 66 months was as great as for the Cleveland group at that age, particularly in view of the relatively high percentage of attacked teeth that had been filled in the Illinois children.

If the Illinois children could have been followed into the school-age range, and if care like that given their temporary teeth up to age 66 months had been continued for a few more years, it is very probable that mortality of their primary teeth would have been well below that experienced by the Cleveland children.

It thus appears that, in respect to the deciduous as well as the permanent dentition, there is a marked inverse relation between the amount of care given teeth reasonably soon after the lesions appear and the amount of tooth mortality suffered later.

DENTAL CARIES EXPERIENCE OF SOME FRESHMAN COLLEGE STUDENTS IN UTAH

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A SURVEY of the dental condition of freshman students was undertaken to obtain basic data on the dental health of a selected group of young Utah people who would represent the various areas of the state. A report by Brown[1] in 1934 on the health of school children in Cache, Box Elder, and Utah Counties included information on the prevalence of dental caries. Of the 892 children examined, 60 per cent had carious deciduous teeth, 70 per cent had carious first molars, and 25 per cent had caries in other permanent teeth. No recent studies giving detailed information on the dental condition of the people of Utah have been made. A preliminary report on the findings from a limited number of college freshmen included in this study has appeared previously.[2]

Similar studies of college freshman students have been reported from Oregon State College[3] and from the University of Idaho.[4] In Idaho more than twofold differences in DMF rate were found in students from different parts of the state.

MATERIAL AND PROCEDURE

The subjects of this investigation were freshman students in 1948 to 1949 and 1949 to 1950 at three colleges or universities of Utah, namely, Utah State Agricultural College at Logan, Brigham Young University at Provo, and Branch Agricultural College at Cedar City.

The dental examination by accredited dentists was done by means of mouth mirror and explorer in good natural light or with additional light from a dental spotlight. The dental caries experience of each student was measured by counting the number of filled teeth, missing teeth because of caries, teeth indicated for extraction, and teeth needing filling. The past and present caries experience for the teeth was calculated as the DMF (decayed, missing, filled) rate. The third molars were excluded in the data reported in this paper because the eruption of third molars had not occurred in the majority of the students.

The U.S.A.C. students were examined by 17 dentists of the First District Dental Society; the B.A.C. students by 4 dentists of Cedar City; and the B.Y.U. students by 15 dentists of the Provo District Dental Society.

A grand total of 1,954 freshman students were examined, 1,102 males and 852 females (787 freshmen at U.S.A.C. in 1948 to 1949 and 506 in 1949 to 1950;

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536 at B.Y.U., and 125 at B.A.C.). The students were classified into the following groups according to their place of residence: Utah, students born and reared in Utah; Idaho, students born and reared in Idaho; Utah-Idaho, students who had lived in both Utah and Idaho; out of state, students who lived elsewhere in the United States; and foreign, students from outside of the United States.

FINDINGS AND DISCUSSION

The average number of DMF teeth and tooth surfaces of the 1,954 freshman students examined are given in Table I. The Utah men and women had 9 to 12 per cent more DMF teeth per person than the men and women of Idaho, out-of-state, or foreign groups in this study (12.3 and 14.2 DMF teeth for the Utah men and women as compared to 9.7 to 10.0 and 10.8 to 13.0 for the other groups). An analysis of variance of the data showed that the differences in DMF teeth between the Utah students and the Idaho and out-of-state students were highly significant. Those students who had lived in Utah and Idaho had DMF values similar to those of the Utah group rather than those of the Idaho group. The number of tooth surfaces for the various groups showed similar relationships.

TABLE I

NUMBER OF DMF PERMANENT TEETH AND TOOTH SURFACES OF UTAH, IDAHO, UTAH-IDAHO, OUT-OF-STATE, AND FOREIGN STUDENTS

	MALE			FEMALE			BOTH SEXES		
LOCALITY	NO.	DMF TEETH	DMF SURFACES	NO.	DMF TEETH	DMF SURFACES	NO.	DMF TEETH	DMF SURFACES
Utah	612	12.3	26.8	523	14.2	31.3	1135	13.2	28.9
Idaho	163	9.7	20.6	154	10.8	22.3	317	10.2	21.4
Utah-Idaho	80	12.2	26.9	35	12.8	28.4	115	12.2	27.4
Out of State	217	10.0	20.5	119	11.5	24.2	336	10.5	21.8
Foreign	30	9.7	22.7	21	13.0	29.2	51	11.1	25.4
Grand Total	1102	11.4	24.6	852	13.1	28.5	1954	12.1	26.3

The dental caries experience of the Utah students was similar to that found at Oregon State College[3] in a study of freshman students (13.2 DMF teeth for Utah and 13.6 for Oregon) and higher than a group of Idaho students examined at the University of Idaho.[4] However, the Idaho students in this study averaged 10.2 DMF teeth as compared to 10.4 for the Idaho study. Five Utah, 10 Idaho, and 9 out-of-state students were found caries-free. These numbers were 0.4, 3.2, and 2.7 per cent of the total in each group as compared to 4.3 per cent of the students in the Idaho study and 0.7 per cent in the Oregon study who were free of tooth decay.

The Utah born and reared students comprised 58.8 per cent of the total number of freshmen of which 54 per cent were men. The age ranged from 17- to 20+. Three students were 16 years old and in the group 20+ about 50 per cent were between the ages of 21 and 24. Approximately two-thirds of the Utah men and women were 18 years old. The age was determined as of the last birthday.

Table II gives a complete summary of the dental caries experience of the Utah born and reared students according to specified age and sex groups. The women had more filled teeth, DMF teeth, and DMF surfaces in each age group than the men. In the 18-year-old group they had slightly more extracted teeth. The women had fewer teeth needing to be extracted, fewer open cavities needing filling, and their gingivae were in better condition than those of the men. The women were taking better care of their teeth as was also the case in the Oregon study.[3] The 18-year-old Utah students had more teeth missing, a similar number of teeth requiring extraction, and similar DMF teeth rates when compared with students in Oregon (1.3 teeth missing, 0.1 teeth needing extraction, and 13.4 DMF teeth for Utah 18 year olds; 0.62, 0.07, and 13.5 for Oregon). In comparison, 18-year-old high school students in Hagerstown, Md.,[5] had 8.7 DMF teeth.

TABLE II

DENTAL CARIES EXPERIENCE OF UTAH BORN AND REARED FRESHMEN STUDENTS
RATES ARE EXPRESSED PER STUDENT BY SPECIFIED AGE AND SEX

AGE AND SEX	NO. STUDENTS	FILLED TEETH	MISSING TEETH	EXTRACTION INDICATED	CAVITIES REQUIRING FILLING	DMF TEETH	DMF SURFACES	CONDITION OF GINGIVAE
17								
Male	28	8.9	0.8	0.0	3.1	11.4	25.7	0.6
Female	30	9.7	0.8	0.0	4.2	12.7	25.3	0.2
Total	58	9.3	0.8	0.0	3.7	12.1	25.5	0.4
18								
Male	353	9.3	1.2	0.1	3.6	12.6	27.3	0.8
Female	348	11.4	1.4	0.0	2.8	14.2	31.4	0.5
Total	701	10.3	1.3	0.1	3.2	13.4	29.3	0.6
19								
Male	139	8.3	1.2	0.1	3.3	11.3	24.2	1.1
Female	99	11.0	1.6	0.0	2.8	14.1	30.8	0.6
Total	238	9.4	1.4	0.1	3.1	12.4	26.9	0.9
20+								
Male	92	9.7	1.8	0.0	2.7	13.0	29.5	0.8
Female	46	12.6	1.7	0.0	2.2	15.4	35.6	0.6
Total	138	10.7	1.8	0.0	2.5	13.8	31.6	0.7

All of the counties of the state of Utah except Daggett were represented in the sample. No one area of the state as represented by these students showed a low incidence of caries attack. The average number of DMF teeth and surfaces of the students from the four counties that had approximately 100 or more students is given in Table III. Differences between counties adjusted for sex were highly significant. The analysis of variance is presented in Table IV. Differences between sexes were also highly significant (adjusted means for the number of DMF teeth were 12.3 and 14.1 for the men and women, respectively). The range in the fluorine content of the water supply[6] for the principal cities in each county is given in Table III.

All the students except the 1948 to 1949 U.S.A.C. students were asked the number of times that they brushed their teeth and their consumption of soft drinks, candy bars, and gum per week. The data for the DMF teeth did not show any correlation with any of the above factors.

TABLE III

NUMBER OF DMF TEETH AND TOOTH SURFACES OF FRESHMEN STUDENTS IN SOME COUNTIES IN UTAH

COUNTY	NO. BOTH SEXES	AGE	DMF TEETH		DMF SURFACES		FLUORINE CONTENT OF WATER† (P.P.M.)
			OBSERVED MEAN	ADJUSTED* MEAN	OBSERVED MEAN	ADJUSTED* MEAN	
Cache	248	17-19	14.8	14.7	33.4	33.2	0.0-0.25
Salt Lake	104	17-19	13.3	13.5	30.0	30.1	0.1-0.40
Box Elder	90	17-19	12.4	12.4	27.2	27.1	0.0-0.30
Utah	172	17-19	12.1	13.2	25.6	26.1	0.0-0.45

*Adjusted for sex.
†Range of fluorine content of water supply for the principal cities of each county.[6]

TABLE IV

ANALYSIS OF VARIANCE—SOME COUNTIES IN UTAH

SOURCES	D.F.	MEAN SQUARES	
		DMF TEETH	DMF SURFACES
Sex (adjusted for county)	1	245.32*	3,643.59*
County (adjusted for sex)	3	238.83*	1,959.69*
Sex and county	3	8.62	62.67
Error	606	26.35	255.97

*Highly significant (P less than .01).

When the source of the home water supply was considered, differences in DMF teeth were small. The students using water from wells averaged approximately 1 less DMF tooth per student for the various groups than students using water from a spring, cistern, or city water. Urban students averaged approximately 1 more DMF tooth per student than the rural students.

SUMMARY

Freshman students at Utah State Agricultural College, Branch Agricultural College, and Brigham Young University were given a dental examination. The Utah men and women, 1,135 of the 1,954 students examined, had 9 to 12 per cent more DMF teeth than the students from Idaho or out of state. These differences were highly significant. Comparisons to results of similar studies in other states were made.

The women had a greater number of DMF teeth, fewer teeth needing to be extracted, and fewer open cavities than the men.

No one area of the state as represented by these students showed a low incidence of caries attack. Differences between the number of DMF teeth and surfaces for the students from four different counties in Utah were highly significant.

We express our appreciation to the following dentists for their services which made this study possible: Drs. G. L. Allen, D. C. Gunnell, C. Haskins, E. L. Larson, W. W. Merrill, H. J. Milligan, N. E. Munk, D. O. Porter, C. C. Randall, C. P. Smith, and I. P. Stewart of Logan; Drs. W. Anderson, W. Bott, B. Harmon, M. R. Peterson, and F. Saito of Brigham City; Dr. R. Mason of Tremonton; Drs. F. Anderson, O. Anderson, J. Beal, and F. Petty of Cedar City; Drs. S. M. Bingham, A. G. Brockbank, T. G. Bullock, A. D. Clark, W. E. Evans, L. N. Hay-

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DENTAL CARIES EXPERIENCE OF A GROUP OF SCHOOL CHILDREN AT OGDEN, UTAH, WITH AND WITHOUT RHEUMATIC FEVER

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A SURVEY of the dental condition of freshman college students in three colleges or universities in Utah[1] showed that the dental caries experience was high. No one area of the state as represented by these students showed a low incidence of caries attack.

Entine[2] in 1949 reported that children with rheumatic fever had 60 per cent more dental caries than did an equal number of normal children. Because of the high incidence of caries attack in Utah students and the possible relationship of dental caries to rheumatic fever, it became of interest to study the dental health of a group of school children equally divided between those who were normal and those having rheumatic fever.

The dental study on these Utah school children was part of a larger study on their nutritional status to determine the relationships between diet and physical well-being and dental health of children with rheumatic fever and normal children. Seven-day diet records, dietary history, medical history and examination, dental examination, dental and bone x-rays, urine analyses for albumin and sugar, blood analyses for hemoglobin, hematocrit, white and red cell counts, sedimentation rate, blood smears and glucose, and serum analyses for ascorbic acid, riboflavin, cholesterol, vitamin A, carotene, alkaline phosphatase, iron, and copper were obtained on the two groups of children. Only the dental findings will herein be reported. Correlations between these findings and the diet records and/or constituents of the blood will be presented later.

PROCEDURE

Dental examinations including posterior bite-wing radiographs for each child were made of the 264 children in the rheumatic fever study by three Ogden dentists. All examinations were made in the dentists' offices. The dental caries experience of the children was expressed in terms of DMF (decayed, missing, filled) teeth for the permanent teeth and DEF (decayed, extracted, filled) teeth for the deciduous teeth.

This study was a part of the Western Regional Research Project on the Nutritional Status of Population Groups in a Selected Area of Utah with the cooperation of the Utah Agricultural Experiment Station. It was financed in part from funds appropriated under the Research and Marketing Act of 1946. The cooperation and assistance of the Bureau of Human Nutrition and Home Economics, and the Utah State Department of Health are acknowledged.

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The children in the rheumatic fever group were from the Ogden, Utah, rheumatic fever clinic and were paired as to age, sex, and economic status with normal children from the same area. Four children of the rheumatic fever group had been apparently active cases within six months before they were subjects in this study. The other children had been active cases prior to that time. The children ranged in age from 5 to 19 years; approximately five-sixths of the subjects of both sexes were in the age group 8 to 16, inclusive. The children were native born and reared in Utah with the exception of 7.6 per cent of those having rheumatic fever and 10.6 per cent of the normal children.

FINDINGS AND DISCUSSION

The dental caries experience including the carious lesions detected by the bite-wing radiographs for the permanent and deciduous teeth of the children in the rheumatic fever study is given by specified age in Tables I and II. Only one girl, aged nine, had experienced no dental caries at the time of the examination. The 5- to 9-year-old children with rheumatic fever had slightly more DMF and DEF teeth, DMF and DEF surfaces affected, and cavities needing filling than did the normal children. However, differences were not significant. Radiograph findings increased the number of teeth and tooth surfaces affected by 10 per cent for either the permanent or deciduous teeth of this age group. The children aged 5 to 9 in the rheumatic group had slightly more deciduous and permanent teeth missing and deciduous teeth needing extraction than did the normal children.

In the age range 10 to 19 the normal children had the greater incidence of dental caries attack, but the differences in the number of DMF teeth, DMF surfaces, or cavities needing filling were not significant. The use of radiographs with this age group increased the number of DMF teeth by 7.2 and 13.6 per cent and the DMF surfaces by 29.9 and 17.8 per cent for the normal and the rheumatic fever groups, respectively. The normal children aged 10 to 19 had fewer teeth missing and teeth needing extraction than did the children with rheumatic fever. The data on these four components comprising the total caries experience of the children for both age groups do not indicate any real difference in caries experience between the two groups of children. Rather, the incidence of caries attack in either group of children appeared to be about equal. This finding is not in agreement with that of Entine[2] who noted 60 per cent more dental caries in rheumatic fever patients than in normal children. However, it should be pointed out that the children in these two studies, which were conducted in different parts of the country, may have varied greatly in their dietary and health habits and economic status. Ninety per cent of the children in Entine's study were from families with incomes below average. This was not the case in the present study as all children with rheumatic fever in the Ogden area who were willing to cooperate were included in the study. The majority of them represented families with average incomes. Also, the number of patients in each study was relatively small for evaluation of data on dental caries experience. These findings indicate a need for further investigations on more patients.

Most of the children had normal gingivae, that is, 86 and 94 per cent of the boys and girls in the rheumatic fever group and 90 and 89 per cent of the normal boys and girls, respectively.

Many of the children were in need of dental care as is indicated by the data in Table I. The average number of teeth per child which needed filling, teeth needing to be extracted, and missing teeth was about equal to or greater than the number of filled teeth in both groups of children. Hadjimarkos and Storvick[3] noted a similar relationship among children of the Willamette Valley of Oregon.

TABLE I

DENTAL CARIES EXPERIENCE OF THE PERMANENT TEETH OF CHILDREN WITH AND WITHOUT RHEUMATIC FEVER
RATES ARE EXPRESSED PER CHILD

AGE	SEX	NO. CHILDREN	FILLED TEETH	MISSING TEETH	EXTRACTION INDICATED	TEETH REQUIRING FILLING	CAVITIES REQUIRING FILLING	DMF TEETH	DMF SURFACES
					Rheumatic Fever				
5-6	M + F	10	0.0	0.2	0.0	0.4	0.4	0.6	1.8
7	M + F	7	0.0	0.0	0.0	1.3	1.6	1.3	1.3
8	M + F	11	1.1	0.4	0.0	3.3	4.6	4.1	8.9
9	M + F	11	2.0	0.1	0.0	1.9	3.0	3.3	6.3
10	M + F	5	1.2	0.2	0.0	2.8	3.6	4.2	6.6
11	M + F	14	2.8	0.3	0.2	5.1	5.6	6.7	12.5
12	M + F	12	4.0	0.8	0.2	3.9	4.8	7.2	17.2
13	M + F	12	6.7	0.6	0.0	5.4	6.9	11.1	23.0
14	M + F	21	4.2	1.0	0.1	7.0	9.1	10.5	23.2
15	M + F	11	7.7	2.9	0.0	5.6	7.1	13.9	35.6
16	M + F	12	5.1	1.8	0.1	7.4	8.5	12.6	30.6
17-19	M + F	6	7.5	1.5	0.5	7.7	9.8	15.2	39.2
5-9	M	21	0.8	0.3	0.0	1.6	2.3	2.4	5.0
5-9	F	18	1.0	0.1	0.0	2.0	2.8	2.5	5.0
5-9	M + F	39	0.9	0.2	0.0	1.8	2.5	2.5	5.0
10-19	M	42	4.4	0.7	0.1	6.1	8.2	9.6	21.9
10-18	F	51	5.2	1.5	0.1	5.6	6.6	10.7	24.6
10-19	M + F	93	4.9	1.1	0.1	5.8	7.4	10.2	23.4
					Normal				
5-6	M + F	10	0.2	0.0	0.0	0.6	0.7	0.8	1.1
7	M + F	8	1.6	0.0	0.0	0.6	0.6	2.2	3.4
8	M + F	10	0.9	0.0	0.0	2.1	3.1	2.8	3.9
9	M + F	11	1.9	0.1	0.0	2.6	3.4	4.1	7.6
10	M + F	7	5.4	0.1	0.0	3.1	3.8	7.1	15.7
11	M + F	11	3.7	0.2	0.1	3.4	4.2	6.3	11.4
12	M + F	13	4.8	0.3	0.0	3.8	4.8	7.8	13.7
13	M + F	12	6.0	1.2	0.2	7.5	10.2	12.9	29.2
14	M + F	21	6.0	0.8	0.1	8.7	11.1	12.7	26.8
15	M + F	13	8.3	1.2	0.0	7.1	9.1	13.8	29.5
16	M + F	16	8.4	1.9	0.0	6.6	7.8	13.6	32.5
5-9	M	21	1.3	0.0	0.0	1.8	2.4	2.9	4.9
5-9	F	18	1.0	0.0	0.0	1.3	1.7	2.2	3.3
5-9	M + F	39	1.2	0.0	0.0	1.6	2.1	2.5	4.1
10-16	M	42	6.4	1.0	0.0	6.3	8.2	11.4	24.6
10-16	F	51	6.3	0.8	0.0	6.2	7.6	11.0	29.0
10-16	M + F	93	6.4	0.9	0.0	6.2	7.9	11.2	27.0

TABLE II

DENTAL CARIES EXPERIENCE OF THE DECIDUOUS TEETH OF CHILDREN WITH AND WITHOUT RHEUMATIC FEVER

RATES ARE EXPRESSED PER CHILD

AGE	SEX	NO. CHILDREN	FILLED TEETH	MISSING TEETH	EXTRACTION INDICATED	TEETH REQUIRING FILLING	CAVITIES REQUIRING FILLING	DEF TEETH	DEF SURFACES
				Rheumatic Fever					
5-6	M + F	10	1.7	0.2	0.6	4.1	4.5	6.1	14.5
7	M + F	7	2.7	1.6	0.3	3.8	4.1	7.6	20.3
8	M + F	11	2.6	2.4	0.3	4.0	5.8	8.2	25.0
9	M + F	11	2.1	1.9	1.2	2.8	3.8	7.0	23.0
5-9	M	21	2.7	1.4	0.4	4.0	5.1	7.6	20.6
5-9	F	18	1.7	1.7	0.8	3.3	4.1	6.7	21.2
5-9	M + F	39	2.3	1.5	0.6	3.8	4.6	7.2	20.9
				Normal					
5-6	M + F	10	3.1	0.2	0.2	3.4	3.5	6.1	12.9
7	M + F	8	3.6	1.0	0.0	3.9	4.4	6.5	16.6
8	M + F	10	2.6	0.7	0.9	4.4	6.4	7.8	21.3
9	M + F	11	2.0	2.4	0.4	2.7	3.5	6.6	22.8
5-9	M	21	3.0	1.2	0.2	3.4	4.2	6.7	18.6
5-9	F	18	2.6	1.0	0.6	3.7	4.3	6.9	18.6
5-9	M + F	39	2.8	1.1	0.4	3.6	4.3	6.8	18.6

The average number of DMF teeth for the combined groups of 15 to 19 years old, 58 children, in the Ogden area was 12.3 as compared to 13.1 for the Utah freshmen students who were 17 to 19 years old.[1] Since the incidence of dental caries experience increases with age, and 90 per cent of the children in this group of the Ogden study were 15 and 16 years old, the DMF rate of these children would have been expected to be lower in this comparison. The caries experience of these Ogden children appeared to be somewhat greater than the average for the Utah college freshmen.

The fluorine content of the water of Ogden and the surrounding area where these children resided averaged 0.15 p.p.m.[4]

The average number of DMF teeth exclusive of x-ray findings, 11.4, for the Ogden children aged 13 to 19 was considerably higher than the 7.1 DMF teeth reported by Klein and Palmer[5] for school children of Hagerstown, Md., aged 13 to 19. The Ogden children aged 12, 13, and 14 had similar numbers of DMF teeth to those reported by Dean[6] for the same ages from cities having less than 0.2 p.p.m. fluorine. The values exclusive of x-ray findings for the 12-, 13-, and 14-year-old Ogden children were 7.2, 10.7, and 10.4, respectively.

Hadjimarkos and Storvick[3] reported an average of 11.8 to 14.4 DMF teeth for 14- to 16-year-old school children in the Willamette Valley and Coastal region of Oregon, and 9 to 11.3 for Central Oregon. The 14- to 16-year-old Ogden children had an average of 11.5 DMF teeth exclusive of x-ray findings.

SUMMARY

Dental examinations including posterior bite-wing radiographs were given 264 school children from the Ogden, Utah, area who were equally divided

between those who were normal and those having rheumatic fever. Differences between the two groups of children aged 5 to 9 or 10 to 19 years were not significant for the number of DMF and DEF teeth, DMF and DEF surfaces, and cavities of the permanent or deciduous teeth.

The caries experience of all of the Ogden children was relatively high when compared with children in other studies in which the same or lower rates of caries experience were reported.

We express our appreciation to Drs. D. A. Buswell, J. O. Douglas, and B. Wolthuis of Ogden for their services which made this study possible. Appreciation is also expressed to Bliss Crandall, U.S.A.C., for his assistance in the statistical analyses.

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EXCITABILITY IN TERMS OF TIME OF THE DENTAL NERVOUS ELEMENTS

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THE development of electrophysiology has permitted the quantitative determination of excitability in a great number of tissues of the human organism which have been studied by diverse methods. Notwithstanding its importance from a diagnostic point of view, the excitability of the dental nervous tissues has been given little attention by physiologists. Most of the clinical methods used to measure excitability, in use in dental practice, are unsatisfactory, excitability usually being expressed in terms of the voltage or current of an electric stimulus. In an earlier paper[13] one of us showed, on theoretical grounds, that these methods have very little diagnostic value and suggested the possibility of obtaining better information on the normality of pulp tissue by studying its excitability in terms of time.

In the present paper an experimental study is reported on the excitability in terms of time of the dental nerve supply. It is the aim of the present work: (a) to obtain information on functional differentiation; (b) to study the possibility of employing the method described below for dental electrodiagnosis. In the following experiments the normal and pathologic chronaxie of the dental nervous elements are measured and their strength-duration function is studied.

METHOD

We employed a condenser chronaximeter. (A resistance of 1,000 has been connected in parallel with the subject at the output of the stimulator.) An experimental control series in which unpolarizable (AgCl-Cl) and polarizable electrodes have been used, showed identical chronaxie values for both types of electrodes. Considering the easier manipulation of the latter, we employed polarizable electrodes. The morphologic variations of the teeth obliged us to use electrodes of special design (Fig. 1), a silver point invested in plasticine being used as the different electrode. The plasticine played the role of an insulator and at the same time held the electrode on the surface of the tooth. The operator kept the ensemble in position by digital pressure. As an indifferent electrode a copper plate was applied to the forearm of the subject by means of cotton moistened in concentrated NaCl solution.

To eliminate the possibility of influencing the duration of the discharge of our condensers by the electric capacity of human skin, we utilized the following control: A condenser of known capacity was discharged through the subject and the course of its discharge photographed on a cathode ray oscillograph (Fig. 2) to measure the electric resistance of the subject. The subject was then replaced by a noninductive resistance of an equivalent value, the condenser dis-

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charged through this resistance, and the oscillographic course of the discharge again registered. Fig. 2 shows both curves of discharge, which if superposed coincide in their full extension. Hence the capacity of the skin is of an order which does not appreciably influence the duration of the condenser discharge of the experiments.

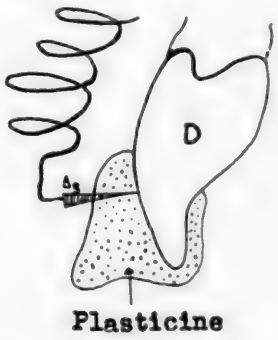

Fig. 1.

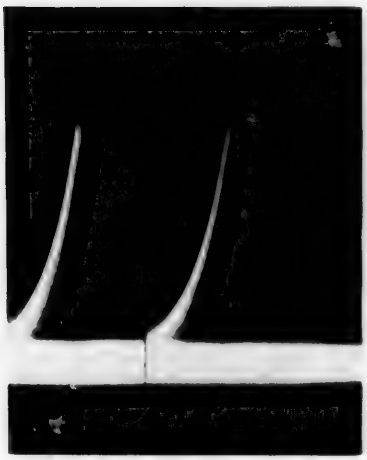

Fig. 2.

The chronaxie measurements are made in the usual way. The dental nervous elements are stimulated with a voltage twice the rheobase and the minimum duration of the stimulating current necessary to cause minimal sensation in the tooth is determined.[11, 13] The sensation perceived was of pressure type.

RESULTS

A total of 164 chronaxie determinations have been made. The results are summed up in Table I. The chronaxies decrease (though not gradually) from

the first incisors to the second upper molars. This difference is statistically significant. Consequently, it can be stated that the chronaxies decrease from the anterior to the posterior teeth in the upper jaw.

If the teeth are classified as uni- and multirooted, the difference appears to be still more noticeable, the former presenting higher values than the latter.

TABLE I

CHRONAXIES OF NORMAL TEETH

TOOTH	CASES	CHRONAXIE (MSEG.)	σ	V.C. (PER CENT)
		Upper Jaw		
First incisor	17	0.258	0.015	5
Second incisor	13	0.199	0.017	8.5
Canine	8	0.214	0.034	15
First premolar	4	0.158	0.022	13
Second premolar	5	0.144	0.015	10
First molar	5	0.152	0.027	17
Second molar	5	0.152	0.024	15
		Lower Jaw		
First incisor	6	0.169	0.049	28
Second incisor	6	0.154	0.019	12
Canine	6	0.203	0.019	24
First premolar	4	0.143	0.057	39
Second premolar	5	0.120	0.040	33
First molar	5	0.123	0.024	19
Second molar	5	0.113	0.007	6

σ (standard deviation of the mean) $= \frac{\sqrt{\Sigma d^2}}{n - 1}$

V.C. (variation coefficient) $= \frac{\sigma \cdot 100}{\text{Mean}}$

The values found in the lower jaw are less than the corresponding ones of the upper jaw (Table I). Nevertheless, this is not statistically significant.

In teeth with pulpitis, 33 chronaxie determinations were performed. Table II shows the results. No significant difference is observed between chronaxies of teeth with normal pulp and those with pulpitis. There was a large dispersion in chronaxies of teeth presenting pulpitis.

TABLE II

CHRONAXIES IN PULPITIS

TOOTH	CASES	CHRONAXIES (MSEG.)	σ	V.C. (PER CENT)
Maxillary first incisor	7	0.210	0.038	18
Maxillary second incisor	8	0.186	0.050	27
Maxillary second premolar	6	0.408	0.166	41
Maxillary first molar	6	0.196	0.080	41
Mandibular first molar	5	0.144	0.057	39
Mandibular second molar	5	0.100	0.032	32

Strength-duration curves were studied, varying the stimulating voltage from a rheobasic value up to approximately 100 volts, and the corresponding utilization times were determined. Fig. 3 shows the characteristic strength-duration curve of the nervous elements of a normal tooth. In order to deal with comparative curves of different teeth and hence obtained under different conditions of resistance, these were reduced to a common scale, using *1* rheobase as unit of the ordinates. Thus, the voltage values were plotted as multiples of *1* rheobase.

Fig. 4 presents a summary of the strength duration functions of the upper teeth. The curve nearest to the ordinates (*A*) belongs to the average function of the second upper molars; curve *B*, to the average function of the upper first incisors. (The magnitude of the arrows represents the standard deviation of the mean.) The functions of the rest of the teeth develop in the area between curves *A* and *B*. Thus it is confirmed in a complete strength-duration curve that the utilization times decrease from the incisors to the molars.

Considering the possibility that, in our experiments, extradental nerve fibers could have been stimulated (i.e., those of the periodontium), a control series of stimulations has been performed in 10 pulpless anterior teeth. Control x-rays were taken to prove removal of the pulp. No response has been observed in any of the 10 control experiments, in spite of the fact that the voltage has been increased to the maximum of our apparatus (approximately 100 volts).

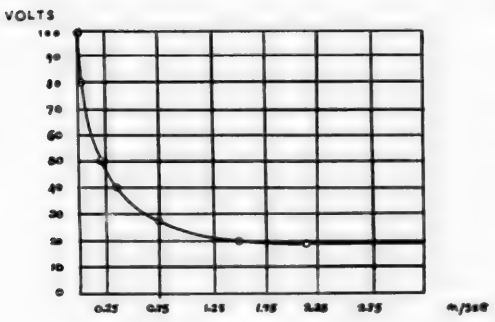

Fig. 3.

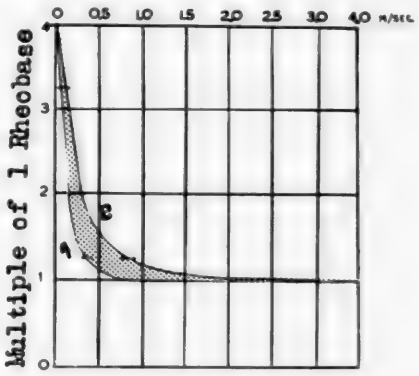

Fig. 4

DISCUSSION

As has already been pointed out, the electrodiagnostic methods commonly used in dental practice are based on measurements of the minimum stimulating voltage. The values of the minimum stimulating voltage (rheobase) were found to be extraordinarily variable if studied in normal homologous teeth of different subjects (Table III). Considerable variations are observed even in the minimum stimulating voltage of the same tooth of a given subject if its determination is made by applying the electrode on different points of the tooth surface (Table IV). Hence, it is evident that little diagnostic value can be expected from methods employing a variable, such as the minimum stimulating voltage, as an index of excitability.

This result had already been anticipated by one of us[13] on theoretical grounds, since the minimum stimulating voltage depends on the resistance of the stimulation circuit, particularly, on the resistance of the enamel, which varies in thickness in different points of the dental surface.

Table IV shows that, notwithstanding the variations of the rheobase due to modifications of the application point of the electrode, the chronaxies of a

TABLE III

RHEOBASE AND CHRONAXIES OF NORMAL HOMOLOGOUS TEETH OF DIFFERENT SUBJECTS

TOOTH	RHEOBASE VOLTS	V.C. OF RHEOBASE (PER CENT)	CHRONAXIE (MSEC.)	V.C. OF CHRONAXIE (PER CENT)
Upper first incisor	20	82	0.185	10
Upper first incisor	33		0.166	
Upper first incisor	14		0.133	
Upper first incisor	25		0.170	
Upper first incisor	81		0.159	
Upper first incisor	22		0.177	
Lower first incisor	4	84	0.133	11
Lower first incisor	20		0.133	
Lower first incisor	22		0.140	
Lower first incisor	5		0.166	

TABLE IV

RHEOBASE AND CHRONAXIE MODIFYING THE APPLICATION POINT OF THE ELECTRODE

TOOTH	RHEOBASE VOLTS	V.C. OF RHEOBASE (PER CENT)	CHRONAXIE (MSEC.)	V.C. OF CHRONAXIE (PER CENT)
Upper first incisor	20	62	0.240	2.8
Upper first incisor	10		0.250	
Upper first incisor	15		0.240	
Upper first incisor	40		0.250	
Upper first incisor	45		0.240	
Upper first incisor	18		0.240	

given tooth are quite constant. The same may be said of chronaxies of homologous teeth belonging to different subjects, with the individual variations already stated (Table III). Thus, it is justified to consider the chronaxie as a good index of the excitability of the dental nervous structures.

Since no significant difference is observed between chronaxies of normal teeth and those presenting pulpitis the use of this method for diagnosis of pulpitis is eliminated. Nevertheless, from a theoretical point of view, it does not necessarily mean that the chronaxie remains invariable during pulpitis. The possibility exists that during the pathologic state, clinically called pulpitis, the nervous structures go through different stages of excitability, i.e., the obtained values will depend on the period of the progress of pulpitis the measurement is made. This hypothesis might explain the much greater dispersion found in chronaxies in pulpitis in comparison with the corresponding ones in normal teeth. A similar phenomenon is observed when excitability is studied in terms of time in a degenerating muscle fiber after section of its motor nerve. In the first stage, the chronaxie of the muscle fiber decreases (½ to ⅓) below its normal value, increasing slowly in the next stage up to approximately 200 times its normal value.[1]

The possibility that responses of extradental nerve structures could have influenced the results is eliminated by the control experiments mentioned above.

The obtained chronaxie values allow the dental nerve fibers to be classified among the elements of high functional differentiation. They are equivalent to those observed in the extensor muscle of the forearm and its corresponding nerves.

The difference we found between anterior and posterior teeth is worthy of a special analysis. In accordance with ideas of Lapicque and others,[11] the velocity of the nerve impulse has an inverse relation with chronaxie, within certain limits. It follows that the significant decrease of the chronaxie may justify the hypothesis that the conduction rate of the intradental nerve fibers in posterior teeth is greater than in anterior teeth. The observed difference may also be explained as due to a phenomenon of spatial summation caused by simultaneous stimulation of a greater number of fibers of the posterior teeth.

SUMMARY

Excitability in terms of time of the intradental nerve supply was studied. The following results are obtained:

The average chronaxie in normal teeth is 0.16 mseg., a value which permits classification of the intradental nerve fibers among the elements of high functional differentiation. The chronaxie decreases from the anterior to the posterior teeth in the upper jaw (0.25 mseg. first incisors to 0.15 mseg. second molars). This difference is statistically significant.

A comparative study of excitability between normal teeth and those presenting pulpitis was made. No significant difference was observed between both groups.

Strength-duration curves of the intradental nerve fibers were determined. They are of simple development in all teeth. The homologous utilization times decrease from the first incisors to the second molars.

A critical experimental study of methods of excitability measurements in common use in dental practice was performed.

The authors wish to express their gratitude for the valuable advice of Prof. Hoffmann and Prof. S. Middleton of the Institute of Physiology and for the cooperation of the Surgical Clinic of Prof. I. Allessandrini who provided the patient-facilities used in this work.

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FIRST PRINCIPLES OF FOUNDATIONAL CAPACITY IN DENTAL INVESTING TISSUES

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CONCENTRATED horizontal force of occlusion causes periodontal damage. While young patients with good periodontal metabolism will tolerate the additional load of horizontal thrust when bridge prostheses are attached to the remaining teeth, and may even experience hypertrophy of the investing tissues as a result of the greater demand, older individuals do not respond as well to the same demand. In most mouths the alveolar process has undergone considerable atrophy and resorption by the fiftieth year, and degenerative changes in the periodontium may come much earlier in life. When teeth are lost, deterioration is accelerated. The mechanics of restorative dentistry may place additional burdens, and periodontal damage may be accelerated. This is evident when cantilever bridgework or clasp-type partial dentures are employed in mouths exhibiting periodontosis.

As a result of this study, we will see that dental investing tissues have variable capacity to tolerate horizontal loads and, further, that it is possible to use dental investing tissues at higher capacity for prosthetic purposes without producing degenerative changes or accelerating the degenerative process. If the investing tissues are intelligently utilized, the life of the remaining teeth will be prolonged. Using investing tissues at maximum capacity has special importance when deterioration of these tissues has occurred either through disease or ageing, and when the remaining teeth must still serve as supports or abutments for prosthetic appliances.

EXPERIMENTAL

A laboratory method was sought to study the rotation of teeth to horizontal forces and the effects of prosthetic structures on the characteristic movements of the abutment teeth to such forces. The investing material should be elastic to simulate the dental investing tissues, and the tooth analogues ought to permit fibrous adhesion to furnish "periodontal membrane."

Korogel was used as an investing material and 2½-inch stove bolts were used as tooth analogues (Fig. 1). The head of the bolt was imbedded in the Korogel together with about 1½ inches of the threads. This level was maintained constantly to rule out complications due to differences in root lengths. The head and threads provided "periodontal attachment" and kept the bolt from rising out of the Korogel during the tests. Two nuts at the upper end of the bolt facilitated placing metal ties or "bridge pontics" between selected teeth for the studies of abutment rotations to horizontal forces. In preparing the specimens, the metal ties were prefabricated by cutting strips 12, 24, 36, 60, 84,

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and 120 mm. from a piece of aluminum stock. The bolts were attached to the ties, and the assembly was submerged to the desired depth in melted Korogel which was then permitted to cool and congeal (Fig. 2).

Horizontal forces were applied through a pulley and a ball chain in order to minimize frictional losses (Fig. 3, *a*). The chain was attached to the individual tooth post or bridge with sticky wax, and the other end supported a weight platform (Fig. 3, *b*). The dependent portion of the chain and platform weighed 25 Gm. To this initial weight, 50, 100, and 150 Gm. were successively applied. For all computations the upper level of weight applied was 175 Gm. The tests were all done well within the range of elastic limit of the Korogel, and no rising or separation took place between the tooth posts and the investing material.

Fig. 1.—Stove bolt assembly with "bridge" ties.

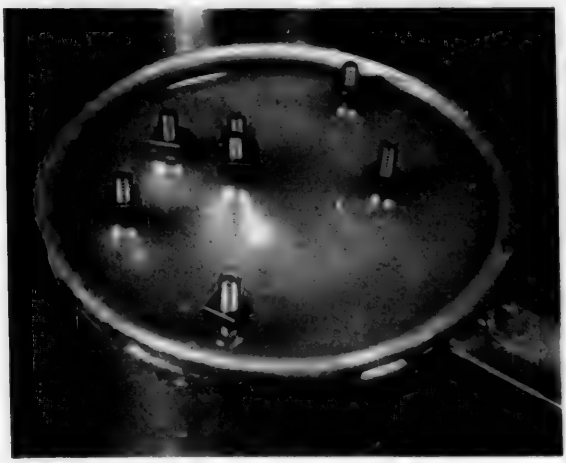

Fig. 2.—Close-up of experimental specimen with bridge ties removed. Posts are arranged to produce bridges with radii of 1, 2, 3, 5, 7, and 10 units length.

A metric ruler was fastened to a support in such a position that a long radius could be used in measuring the rotation of each test post. No attempt was made to measure the rotation in degrees. For some of the tests a rifle

sight was employed to obviate parallax in reading the rotations. Later a fine wire pointer was used. The radius of the pointer used in denoting the rotation was maintained at constant length for all data reported.

DISCUSSION

The author has observed over a long period of time that cantilever bridges (with a lug at the secondary abutment) exhibit more mobility to horizontal thrust than fixed bridges (fixed at both ends). The stability of the latter is out of proportion to the additional support of the second abutment. This can be explained by the fact that the second case takes advantage of leverage and thereby gains mechanical advantage. The individual tooth (Fig. 4) turns on an axis of rotation, *a*, to horizontal force, *b*. Only the periodontal fibers on the tensile half of the root from the axis of rotation occlusalward are resisting the force because the fibers on the other side are being compressed. Even the resistance of the tensed fibers becomes progressively less as points are taken closer and closer to the axis of rotation because the displacement of the tooth is proportional to the distance from the axis. The same relations hold in reverse order on the opposite side of the tooth from the axis of rotation to the root end. However, since the axis of rotation is in the apical third of the root of the tooth, the periodontal membrane is under disadvantage to offer much resistance to horizontal force in this region.[1]

Fig. 3.—Experimental assembly with test being run on individual post No. 4.

Multirooted teeth are more capable of resisting horizontal displacement. Fig. 5 illustrates how a lower molar is displaced by distal (or mesial) thrust. On distal thrust (Fig. 5, *b*) the mesial root goes up, the distal root moves down, and rotation takes place on a center (Fig. 5, *a*) lying in the bone septum between the two roots. In other words, a certain amount of translation of the roots occurs and the periodontal fibers of both roots come more completely into play.

The significant difference between the two instances has been unrecognized. Recognition of the fact provides a means of intelligently utilizing investing tissues at greater capacity.

The rotational property here described is not peculiar to teeth. Figs. 6 and 7 illustrate the same conditions occurring in the laboratory specimen. When Fig. 7, *a* moves up to horizontal push, *b* moves down and rotation occurs upon a center, *c*, in the Korogel between the bolts.

All human dentitions exhibit arch form. When any two teeth are firmly united together as by splinting inlays or fixed bridgework, the foundational stability of both teeth is greatly enhanced because rotation in response to horizontal thrust must take place on a center between the roots of the two teeth. One tooth moves down under the thrust, the other up, depending upon which side of the center it happens to be. In certain of the mandibular movements, the foundational stability of such teeth increases arithmetically, in other movements the mechanical advantage of leverage is gained. When posterior teeth of both sides of either the mandible or the maxilla are firmly united in a fixed prosthesis, the center of rotation is at a considerable distance from the abutments, the translational effect of the roots becomes more pure, and the periodontal membrane of each entire root is called into play. The experimental data substantiate this view.

Fig. 4.

Fig. 5.

Fig. 4.—Schematic model of single-rooted tooth to demonstrate axis of rotation, *a*; to horizontal thrust, *b*.

Fig. 5.—Model of multirooted tooth demonstrating axis of rotation in bone septum between roots.

From a practical standpoint, the significance of this finding is that prostheses which utilize firmly fixed abutments separated by long distances should be very stable to horizontal forces in mastication. A fixed bridge which unites both canines and a molar tooth should be very stable both to buccolingual as well as anteroposterior forces. Since such constructions are seldom undertaken due to the presence of anterior teeth, precision attachment-type bridge-

work, which approaches closest to the positive coupling of a fixed bridge, is the next best choice. The torque of a partial denture with cantilever saddle extension over one edentulous posterior region would be effectively and safely tolerated by the canine and molar of the opposite side, provided the case is rigid enough across the arch.

Fig. 6.—Individual post in Korogel turns on a hinge axis of rotation like single-rooted tooth. Center of rotation at *a*.

Fig. 7.—Abutments of "bridge" turn on an axis of rotation in Korogel between the "roots" like a lower molar.

RESULTS

The rotation of post No. 4 *(Fig. 3)* to 175 Gm. of loading was 12 millimeters. To similar loading the rotation of each post is shown in Table I. The variance in rotation is due to two factors: first, proximity of the post to the side of the pot, second, shrinkage of the Korogel which left some posts a little less supported than others. The order of values is, however, quite satisfac-

TABLE I

POST NUMBER	DISPLACEMENT OF POINTER (MM.)
1	15.0
2	12.5
3	11.8
4	12.6
5	14.0
6	13.0
7	11.5
7	89.8
	12.8 mm. average displacement

tory, with an average of 12.7 mm. rotation, whereas the mean value would probably be nearer 12.

When the posts were united by bridges, a load of 175 Gm. applied at right angles or 90 degrees to the bridge span produced readings as shown in Table II. With minor variation the rotation of the double posts is about half that of the individual posts, and this value is constant regardless of whether the abutments are close together or far apart.

TABLE II

POST NUMBER	MM. BETWEEN CENTERS	DISPLACEMENT OF POINTER (MM.)
4 & 5	12	8.0
3 & 4	24	6.0
2 & 3	36	5.5
1 & 2	60	6.0
1 & 6	84	6.0
1 & 7	120	6.0

When the load is applied in line with the abutments, that is, at 0 degrees, the distance between centers of the abutments is an important factor. At 12 mm. centers, the abutments rotated through a pointer displacement of 10 mm.; at 120 mm. centers, the displacement was $\frac{7}{8}$ mm. Obviously the increased spacing of the abutments makes for stability. Fig. 8 and Table III give intermediate values. Since rotation occurs upon a center between the posts, the stability of the foundation or its resistance to rotation varies directly as the radius.

TABLE III

	UNITS					
	1	2	3	5	7	10
mm. between post centers	12	24	36	60	84	120
mm. rotation of pointer at 175 Gm. load	10	$5\frac{3}{4}$	$3\frac{3}{4}$	$2\frac{4}{5}$	$1\frac{1}{2}$	$\frac{7}{8}$
Calculated rotation	10	5	$3\frac{1}{3}$	2	$1\frac{3}{7}$	1

The angle of application of the horizontal force also is important to setting up foundational stability. When the horizontal load is applied at 60 degrees, 45 degrees, and 30 degrees successively to the span of the 36 mm. bridge, the readings of the pointer to 175 Gm. of load become progressively reduced and, therefore, the foundation becomes more stable (Fig. 9). The two extremes have already been considered.

When the test is repeated, using abutments at 120 mm. centers, stability of the foundation increases as the angle of application of force is reduced (Fig. 10). It should be noted also that since the abutments are separated by a long distance, the radius of rotation is great, and the stability values rise rapidly as the angle of application of force is reduced.

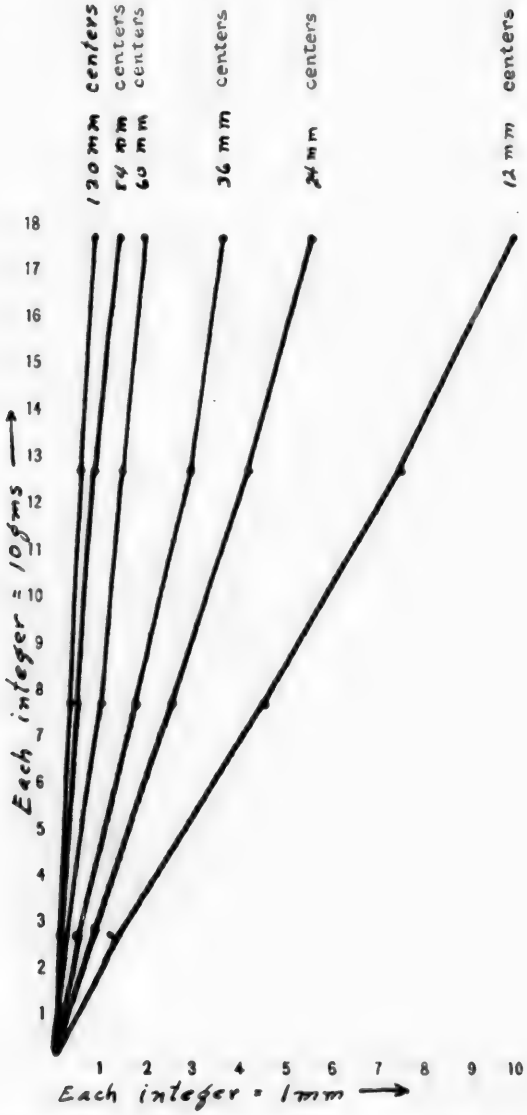
120 mm centers
96 mm centers
60 mm centers
36 mm centers
24 mm centers
12 mm centers
Each integer = 10 gms
Each integer = 1 mm
1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Fig 8.—Graph of rotations of bridges 1, 2, 3, 5, 7, and 10 units in length to horizontal loading applied at 0 degrees.

COMMENTS

By clinical observation of the masticatory cycle, one would conclude that when the lower teeth move from "working bite" to centric, the mandible shifts bodily sideways. That this is not the case can be ascertained by taking a "Gothic arch" tracing. The lines drawn on the photograph (Fig. 11) indicate the reason for the geometry of the Gothic arch. It must be obvious, too, that for right-sided chewers the tangent B_2 to arc B_1 is the direction of the horizontal force playing against the teeth or any prosthetic device of the upper teeth in working bite.

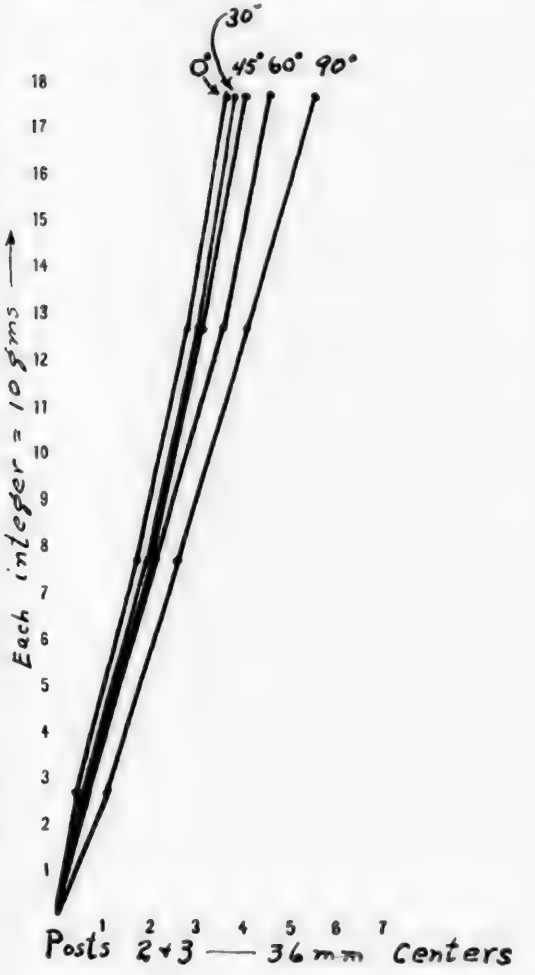

Fig. 9.

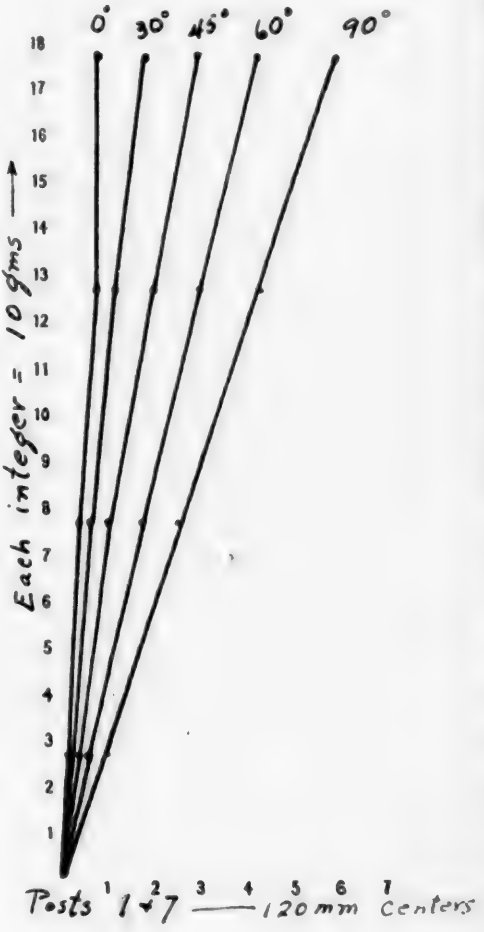

Fig. 10.

Fig. 9.—Graph of rotations of 36 mm. bridge to horizontal loading applied at 90 degrees, 60 degrees, 45 degrees, 30 degrees, and 0 degrees.

Fig. 10.—Graph of rotations of 120 mm. bridge to extreme and diagonal horizontal loading as designated.

If friction were the only factor to consider, the movement of the mandible from working bite to centric would thrust the upper posterior teeth lingually. But since the action of the mandible is exerted through points on the lower teeth in contact with cuspal inclines on uppers, the upper teeth are thrust buccally. This is particularly true because the contact of the teeth takes place on surfaces lubricated by saliva. The horizontal force component of the vertical bite will vary depending upon the pitch of the occlusal cusps being

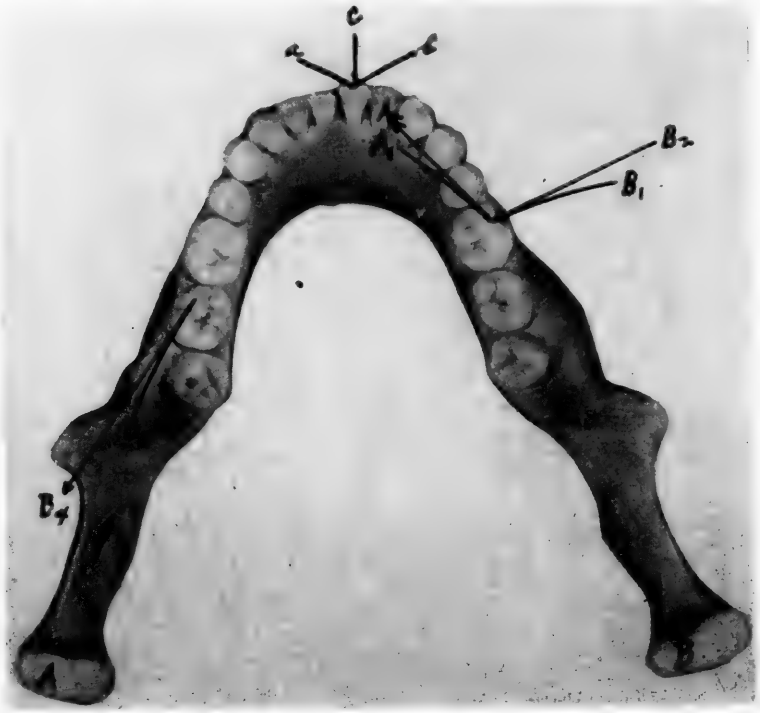

Fig. 11.—(1) When condyle *A* moves from left protrusive to centric, rotation of the mandible occurs on *B* as center and arc *b* of the Gothic arch is scribed. (2) When condyle *B* moves from right protrusive to centric, *A* is center of mandibular rotation and arc *a* of Gothic arch is scribed. (3) When both condyles move into protrusive, protrusive recording *c* is scribed. (*Note:* These markings are just reversed for the conventional Gothic arch tracing because the scriber is usually attached to the upper jaw.) (4) In "working bite" when *A* moves from its protrusive position to centric, mandibular rotation occurs on *B* as a center, arch B_1 is scribed which has for its tangent B_2. (5) If the mandible "overshoots" centric, *B* moves into protrusive, *A* becomes the center of mandibular rotation, and arc A_1 is scribed which has for its tangent A_2. (6) In "balancing bite" (right-sided chewers) *B* is the center of mandibular rotation when *A* moves from protrusive to centric and arc B_3, which has for its tangent B_4, is scribed by this movement.

greater for steeply inclined cusps than flat cusps, and because of the magnitude of the vertical forces the horizontal components may be very significant. In the act of mastication, however, this situation does not exist. When heavy chewing forces occur, food is compressed between the teeth before the cusps puncture through to make contact with the opposing cuspal planes. If the food is tough, the horizontal force produced by the movement of the mandible when moving from working bite to centric must be very significant also. Since the horizontal force component of the vertical bite is in opposite phase with the hor-

izontal movement of the mandible, the two forces tend to cancel each other and the significant force is axial or vertical.

Since the upper teeth are stationary, the horizontal force of mandibular movement which acts upon the lower teeth is in opposite phase to the upper. It is interesting but not comforting to note that the foundational capacity of the investing tissues is least during the working-bite stroke of the masticatory cycle because the horizontal force is applied at almost 90 degrees, as can be determined by tangent B_2 (Fig. 11). The cancellation of the horizontal forces just mentioned, however, mitigates the severity of the situation.

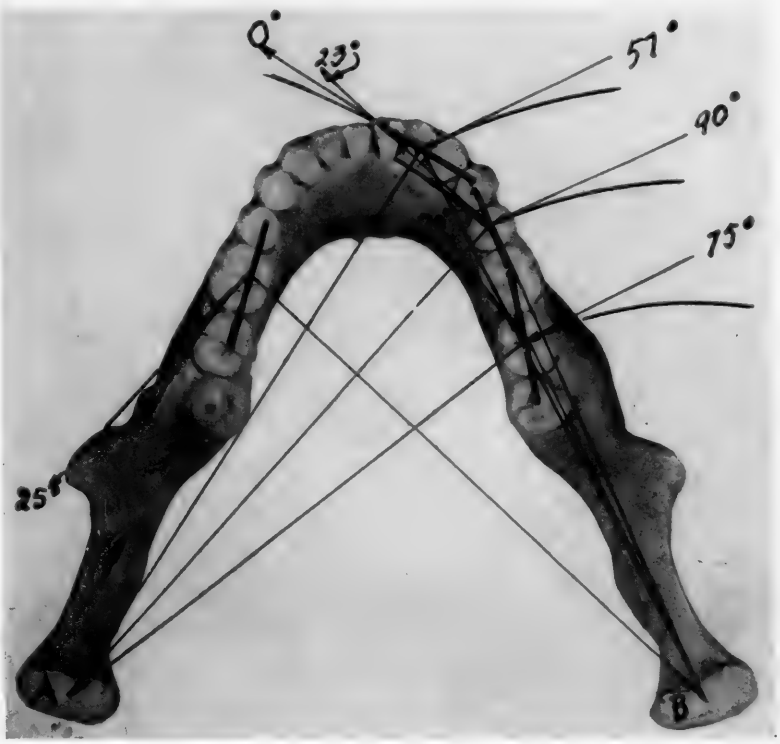

Fig. 12.—Angles of application of horizontal forces when the mandible moves from working bite to centric.

If the chewing stroke does not stop exactly at centric but the mandible continues on past this position a short distance, the head of the condyle B leaves the glenoid fossa to come forward, and rotation begins on A as center. The tangent A_2 to arc A_1 indicates that horizontal force is applied in a direction approaching the anteroposterior. The teeth collectively resist this force through interproximal contact. It should be observed that horizontal force in this direction is well resisted by fixed bridgework because the angle of application now approaches 0 degrees. Since the movement of the lower teeth at this time is in an anterior direction, friction will tend to seat upper cantilever bridges supported by a lug at the anterior abutment. By the same token,

since the horizontal force on the lower teeth will be equal but opposite in phase, lower cantilever bridges supported as above may be pulled out of lug contact with the secondary when tough or fibrous food exists between the teeth, and the primary abutment will take all the horizontal thrust.

For right-sided chewers the horizontal force on the "balancing side" is directed not buccolingually but anteroposteriorly. In this instance the mandible is turning on a center at B. The tangent B_4 to arc B_3 indicates that, if abutments are united in a fixed bridge on this side, foundational capacity of the abutment teeth will be exploited to the fullest because the horizontal force is applied through an angle now again approaching the 0 degree limit. Since the lower teeth are moving posteriorly during this masticatory stroke, long cantilever construction in upper bridges is inadvisable because, unless perfect intercuspation exists, transitional contact of cusps may cause the cantilever to rise out of its seat in the secondary abutment during the milling action and the primary abutment will take all of the powerful horizontal masticatory force directed distally.

Similar mandibular and foundational relations can be obtained for left-sided chewers except that the directions of forces concerned will be reversed.

There is improvement in the capacity of investing tissues to tolerate the horizontal load when abutments anterior or posterior to the premolar-first molar region are firmly united in bridgework. This is illustrated in Fig. 12. The heavy lines across the centers of the teeth denote fixed bridgework with an abutment at the terminus of each line. The figures denote the angles of application of force at the center of each bridge span as the mandible moves from working bite to centric and somewhat past centric.

SUMMARY

To study the characteristic movements of teeth to torque and the effects of prosthetic structures on such movements, stove bolts were imbedded in masses of Korogel to simulate teeth in investing tissues. Single teeth as well as multiple teeth supporting bridgework may be studied for rotational effects to horizontal forces or eccentric loading. Variables relating to size and lengths of the root portion imbedded in the investing material can be kept at laboratory constancy and with simple measuring devices a close quantitative analysis may be reached to direction and degree of rotation of teeth to horizontal forces. Fixation of abutments alters the characteristic simple hinge rotation of the individual teeth to produce larger arcs of rotation. Rotation of a structure with fixed abutments occurs upon a center lying in the bone between the abutments. The abutments move bodily up or down depending upon which side of center they happen to be. Mechanical advantage is gained by the larger orbit of rotation. Foundational stability varies directly as the radius and is further dependent upon the angle of application of the horizontal force.

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MUSICAL THERAPEUTICS

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INTRODUCTION

MOST patients approach the dental chair and operating table with reluctance, ranging from slight uneasiness to marked apprehension manifested by syncope. Some factor other than trauma may cause a patient to become unconscious after a minor manipulation. The two extreme types of reactors are the passive and the agitated, between which there are all variations. It is probable that the patient's personality (patterned modes of adaptation) is an important determinant in these various responses. This apprehensive state or anticipation syndrome has posed a problem difficult to solve. Anticipation syndrome may be the more descriptive term because ". . . interestingly, the perception of pain is generally reduced after prefrontal lobotomy, for the advance dread is removed, and the magnifying glass of anticipation is out of service. In other cases which have been reported, drug addicts suffering excruciating pain during deprivement have demonstrated little evidence of such suffering after a similar operation."[1]

In the past, most approaches to the patient problem have been in the form of diagnosis and treatment of organic disease. Recently the term psychosomatic medicine has been coined to refer to a point of view which regards all illness, even health, as having a psychological, as well as physical aspect, in both its cause and manifestations. We have become very much aware of the importance in treating "the whole person" as an integrated functioning unit, recognizing psychologic factors along with structure.

In an attempt to relieve the anxiety and fear and to reassure the patient, many methods and aids have been tried, and one among these is music. Analyzing the powers of music one should keep in mind that music has always been an important factor in the instinctual, emotional, cultural, and spiritual life of people, and as such has exercised a sort of therapeutic influence. Music, even more than the spoken word, lends itself as a therapy because it meets with little or no intellectual resistance, and does not need to appeal to logic or initiate action. It is more subtle and primitive and, therefore, its appeal is wider and greater.[2]

Best[3] obtained very favorable results in dentistry by supplying music via earphones built into the headrest. Brown, Livingston, and Willard[4] reported the use of "silent music" to soothe surgical patients. They contended the mental diversion made the patient's stay in the operating room seem shorter and was a definite aid in allaying nervousness and apprehension. They reported that the selection of the correct type of music is very important. It must be a melody that will calm and soothe the patient and not excite or

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stimulate him, yet it must hold his attention. There are many reports of the use of music in dentistry and medicine and today it appears more popular than ever.

As it is difficult to measure mechanically the emotional state of a patient, a questionnaire was decided on to ascertain the qualitative effect of music in overcoming the "anticipation syndrome."

MATERIAL AND METHOD

In an attempt to supply the different types of music and programs desired by the patient, a wire recorder, radio, and record player were used. From a central location music was supplied to three dental chairs. In one method, the earphones were located in the headrest, and in the second a stethoscope type of earphone was used. An adjustable volume box was located on the chair within easy reach of the patient's hand, so that the patient could adjust the volume himself during the operation.

A total of 205 patients took part in this study. The types of work were roughly divided into operative and oral surgery. All 139 of the surgical patients were premedicated and received local anesthesia. All 66 operative patients received local anesthesia. All types and ages of patients were represented in the survey; however, the majority were males in their twenties. The patient was interviewed prior to any work. He was questioned about his previous dental experiences, and an attempt was made at that time to estimate the amount of pain that might be encountered and classify him as to his emotional state. This was accomplished through the questions listed in Table I and consideration of the patient's general condition. It was considered that a passive-type patient in need of a minor operation would not require the same amount of premedication as the extremely apprehensive patient who was to undergo a major procedure.

TABLE I

HISTORY	YES	NO
1. Did dentistry scare you as a child?	61	144
2. Do you get a little nervous about it?	107	98
3. Have you ever had an unpleasant experience with a dentist?	63	142

The estimated amount of pain, using the average patient as a norm, was recorded pre- and postoperatively. This was used in determining the correct amount of premedication to administer.

Patients were classified into one of four groups (Table II). A greater percentage of patients were classified as passive and mildly apprehensive post-

TABLE II

CLASSIFICATION OF PATIENT TYPE	PREOPERATIVE	POSTOPERATIVE
Class I Passive	68	104
Class II Mildly apprehensive	100	88
Class III Extremely apprehensive	36	13
Class IV Pathologic	1	0

operatively than preoperatively. Although this was a gross classification, it does illustrate the possibility of overcoming apprehension due to the treatment instituted.

The intelligence level for selecting music proved, as in other surveys, that personal tastes varied generally with the amount of education received by the individual, that is, more patients with advanced training preferred classical music; there were, however, exceptions. The patient was asked if he liked music. When possible, the requested type of music was supplied. Prior to placing the earphones, the small volume control box was pointed out to the patient and instructions given as to increasing or decreasing the sound intensity.

TABLE III
MUSIC PREFERENCE

	NO.
Like music	198
Do not like music	7
Type:	
Modern	140
Classical	59
Hillbilly	6
Quiet and soft	181
Loud and exciting	24

The patient was then prepared and the operation carried out. After the work was completed, the patient was questioned as to (1) whether he listened to the program, (2) whether he enjoyed it, (3) whether he would have preferred a different type, and (4) whether he believed it helped him.

TABLE IV
REACTION TO MUSIC OF 205 PATIENTS

	PER CENT
Did listen	94
Did enjoy program	91
Preferred different type	9
Was a definite help	88

There seemed to be an almost universal love for music. The majority desired popular music, with individual preference for the quiet and soft, sweet lyrical type.

SUMMARY

The purpose of this study was to determine the efficacy of music in reducing and alleviating the anticipation syndrome. It was found that approximately 52 per cent of the patients interviewed admitted apprehension, with 49 per cent classified mildly and 17 per cent extremely apprehensive. Even the passive types admitted a slight uneasiness, demonstrating a higher percentage of patients who were afflicted with the anticipation syndrome, while 31 per cent had had an unpleasant experience with a dentist.

The preponderance of feeling (88 per cent) by the patients that they were helped, i.e., their apprehension alleviated by the diversion of music during oral procedures, is conclusive. This should point the way and stimulate

greater effort to investigate the problem of facilitating the administration of painful or possibly painful and feared types of treatment, by means of utilizing specific psychologic treatment concurrently with surgery and drugs.

CONCLUSIONS

It was our general impression that the patients fell into three classes:

1. Those patients who were totally indifferent or stoical.

2. The majority, who were benefited or had their attention diverted sufficiently to feel they were helped.

3. Those patients so extremely apprehensive, not necessarily pathologic, that a general anesthesia would have been the better procedure.

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THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
CHICAGO SECTION

SCIENTIFIC PROGRAMS FOR 1951-1952

COMPILED BY G. B. DENTON

AT THE five meetings of the Chicago Section held during the year 1951 to 1952, the following nineteen papers were presented:

October 26, 1951.—Implantation of Certain Chemotherapeutic Agents in Impacted Lower Third Molar Sockets. Eli Olech, College of Dentistry, University of Illinois.

Preliminary Investigations on the Local Implantation of Aureomycin in Lower Bicuspid and Molar Extraction Wounds. Richard L. Verbic, M.S.D., Northwestern University Dental School.

A Universally Acceptable Definition of Dental Caries. Frank J. Orland, Zoller Clinic, University of Chicago.

Phosphate Assimilation and Dental Caries. Thomas B. Coolidge, Zoller Clinic, University of Chicago.

November 30, 1951.—Histological Report on Condylar Regeneration in the Rat. Joseph R. Jarabak, Northwestern University Dental School.

Surgical Reduction of Mandibular Condylar Fracture. Orion Stuteville, Chicago College of Dental Surgery.

Effect of Sodium Copper Chlorophyllin on Growth and Acid Production of Some Oral Bacteria. Stewart A. Koser and Eleanor A. Hodges, University of Chicago.

A Study of the Innervation of the Maxillary Teeth as Derived From the Maxillary Nerve. William L. McDaniel, Northwestern University Dental School.

January 25, 1952.—A Cephalometric Radiographic Investigation of the Skeletal Pattern of a Specific Dental-Facial Anomaly. W. E. Gerber, Northwestern University Dental School.

Effect of Antabuse Upon Dental Changes in Vitamin E Deficient Rats. J. J. Pindborg, Royal Dental College of Denmark, Copenhagen.

Influence of Osmotic Pressure in the Carious Process. G. W. Rapp, Loyola University Dental School.

Dental Research in South America. L. S. Fosdick, Northwestern University Dental School.

April 25, 1952.—Histochemical Aspects of Irradiated Bone. Marvin Burstone, School of Dentistry, University of Illinois.

A Radiographic Study of the Velo-Pharyngeal Closure and the Linguo-Velar Relationship in Certain Vowel Sounds. Robert L. Williams, M.S.D., Northwestern University Dental School.

Growth at the Frontonasal Suture of the Rabbit: A Gross and Serial Roentgenographic Study. A. J. Selman, School of Dentistry, University of Illinois.

The Odontoblast. Harry Sicher, Loyola University School of Dentistry.

May 23, 1952.—A Résumé of the Evanston Dental Caries Study. J. R. Blayney, Zoller Memorial Clinic, University of Chicago.

Changing Philosophies in Cleft Palate Rehabilitation. T. M. Graber, Northwestern University Dental School.

Preliminary Report on the Solubility of Bone in Alizarin Solution. Part I. R. Q. Blackwell, Department of Chemistry; Part II. J. R. Jarabak, Department of Anatomy, Northwestern University Dental School.

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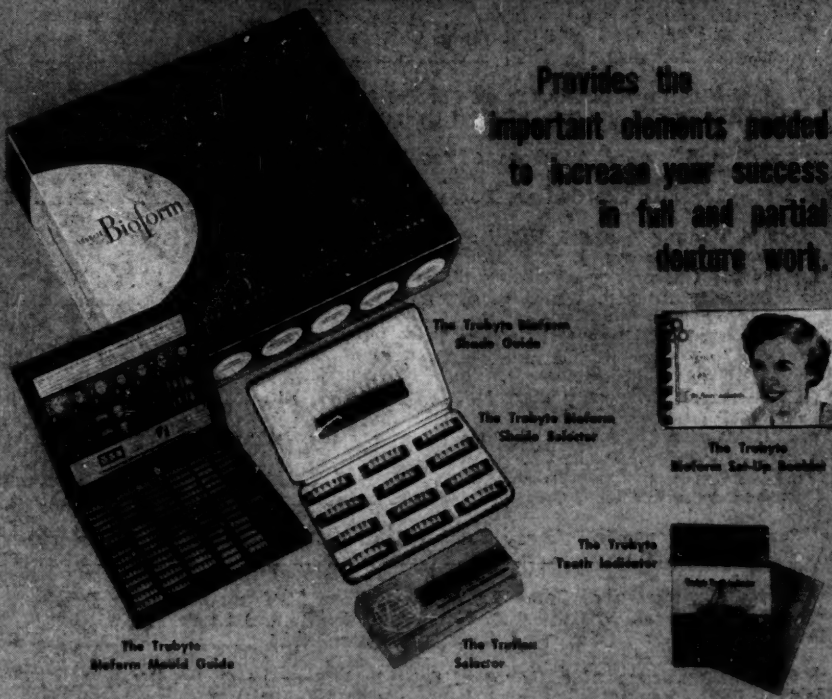
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